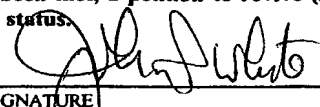


JC13 Rec'd PCT/PTO 01 NOV 2001

FORM PTO-1390 (REV. 9-2001)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 59138-B-PCT-US/JPW/FHB	
<b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b>				U.S. APPLICATION NO. (If known, see 37 CFR 1.5 <b>10/018192</b> NOT YET KNOWN	
INTERNATIONAL APPLICATION NO. PCT/US00/12065		INTERNATIONAL FILING DATE 3 May 2000		PRIORITY DATE CLAIMED 3 May 1999	
TITLE OF INVENTION DNA ENCODING SNORF36a AND SNORF 36b RECEPTORS					
APPLICANT(S) FOR DO/EO/US SYNAPTIC PHARMACEUTICAL CORPORATION					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below. 4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) a. <input checked="" type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). (courtesy copy) b. <input checked="" type="checkbox"/> has been communicated by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). a. <input type="checkbox"/> is attached hereto. b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (unsigned) 10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). Items 11 to 20 below concern document(s) or information included: 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment. 14. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment. 15. <input type="checkbox"/> A substitute specification. 16. <input type="checkbox"/> A change of power of attorney and/or address letter. 17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825. 18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). 19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 20. <input checked="" type="checkbox"/> Other items or information: Courtesy copy of Notice Informing Applicant of the Communication of the International Application to the Designated Offices, PCT Request, PCT Demand, International Preliminary Examination Report, Renewed Petition Under 37 C.F.R. 1.181 and Petition For Three Month Extension of Time Under 37 C.F.R. 1.136(a), Statement in Accordance with 37 C.F.R. 1.821(f), and Small Entity Declaration from U.S. parent application, 1 loose set of formal drawings, and an Express Mail Certificate of Mailing bearing Label No. EK 873 630 962 US dated November 1, 2001					

U.S. APPLICATION NO. (if known, see 37 CFR 1.51) not yet known <b>8192</b>		INTERNATIONAL APPLICATION NO. <b>PCT/US00/12065</b>		ATTORNEY'S DOCKET NUMBER <b>59138-B-PCT-US/JPW</b>	
21. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... <b>\$1040.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... <b>\$890.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... <b>\$740.00</b>  International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... <b>\$710.00</b>  International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... <b>\$100.00</b> <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>CALCULATIONS PTO USE ONLY</b>	
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				<b>\$ 710.00</b>	
<b>CLAIMS</b>	<b>NUMBER FILED</b>	<b>NUMBER EXTRA</b>	<b>RATE</b>	<b>\$</b>	
Total claims	18 - 20 =	0	x <b>\$18.00</b>	<b>\$ 0.00</b>	
Independent claims	3 - 3 =	0	x <b>\$84.00</b>	<b>\$ 0.00</b>	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				<b>+ \$280.00</b>	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				<b>\$ 710.00</b>	
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				<b>\$ 355.00</b>	
<b>SUBTOTAL =</b>				<b>\$ 355.00</b>	
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				<b>\$ 0.00</b>	
<b>TOTAL NATIONAL FEE =</b>				<b>\$ 0.00</b>	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property +				<b>\$ 0.00</b>	
<b>TOTAL FEES ENCLOSED =</b>				<b>\$ 355.00</b>	
				<b>Amount to be refunded:</b>	<b>\$</b>
				<b>charged:</b>	<b>\$</b>
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>355.00</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>03-3125</u> . A duplicate copy of this sheet is enclosed. d. <input type="checkbox"/> Fees are to be charged to a credit card. <b>WARNING:</b> Information on this form may become public. <b>Credit card</b> <b>information should not be included on this form.</b> Provide credit card information and authorization on PTO-2038.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: John P. White, Esq. Reg. No. 28,678 Cooper & Dunham LLP 1185 Avenue of the Americas New York, New York 10036					
				 SIGNATURE	
				<u>John P. White</u> NAME	
				<u>28,678</u> REGISTRATION NUMBER	

Dkt. 59138-B-PCT-US/JPW/FHB

IN THE UNITED STATES DESIGNATED ELECTED OFFICE (DO/EO/US)

Applicants : Beth E. Borowsky, et al.  
International  
Application No. : PCT/US00/12065  
Filed : 3 May 2000  
For : DNA ENCODING SNORF36a AND SNORF 36b  
RECEPTORS

1185 Avenue Of The Americas  
New York, New York 10036  
November 1, 2001

Assistant Commissioner for Patents  
Washington, D.C. 20231  
Attn: DO/EO/US

Sir:

PRELIMINARY AMENDMENT

Applicants request that the following amendments be made in the above-identified application:

In the Claims:

Please cancel claims 15-18, 20-35, 37-46, 48-57, and 59-165 without disclaimer or prejudice to applicants' right to pursue the subject matter of these claims in a future continuation or divisional application.

Beth E. Borowsky, et al.  
 U.S. Serial No. : Not Yet Known  
 (§371 National Stage Application of  
 PCT/US00/12065, filed 3 May 2000)  
 Filed: Herewith  
 Page 2

**REMARKS**

This application is a §371 national stage of PCT International Application No. PCT/US00/12065, filed 3 May 2000, designating the United States of America, which claims priority of U.S. Serial Nos. 09/518,914, filed March 3, 2000, and 09/303,593, filed May 3, 1999. Accordingly, the parent application, PCT International Application No. PCT/US00/12065, is pending today in the United States of America pursuant to 35 U.S.C. §363, and the subject §371 national stage application is co-pending therewith in fulfillment of the provisions of 35 U.S.C. §120.

Sequence Listing

The Sequence Listing in the subject application is identical to the Sequence Listing in the parent of the subject application, namely PCT International Application No. PCT/US00/12065, filed May 3, 2000. Applicants attach herewith copies of the paper copy of the Sequence Listing (20 pages) and the Statement In Accordance With 37 C.F.R. §1.821(f) which were filed with PCT International Application No. PCT/US00/12065 on May 3, 2000. In accordance with 37 C.F.R. §1.821 (e), please use the computer readable form filed in PCT International Application No. PCT/US00/12065 on May 3, 2000 as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant application.



1003.21.9210/018192

JC13 Rec'd PCT/PTO 01 NOV 2001

1795/59138-B-PCT-US/JPW/FHB

**Application  
for  
United States Letters Patent**

**To all whom it may concern:**

**Be it known that**

Beth E. Borowsky, et al.

**have invented certain new and useful improvements in**

DNA ENCODING SNORF36a AND SNORF36b RECEPTORS

**of which the following is a full, clear and exact description.**

35/pv

JC13 Rec'd PCT/PTO 01 NOV 2001

DNA ENCODING SNORF36a AND SNORF36b RECEPTORS5 BACKGROUND OF THE INVENTION

This application is a continuation-in-part and claims  
priority of U.S. Serial No. 09/518,914, filed March 3, 2000,  
a continuation-in-part of U.S. Serial No. 09/303,593, filed  
10 May 3, 1999, the contents of which are hereby incorporated  
by reference into the subject application.

Throughout this application various publications are referred  
to by partial citations within parenthesis. Full citations  
15 for these publications may be found at the end of the  
specification immediately preceding the claims. The  
disclosures of these publications, in their entireties, are  
hereby incorporated by reference into this application in  
order to more fully describe the state of the art to which  
20 the invention pertains.

Neuroregulators comprise a diverse group of natural products  
that subserve or modulate communication in the nervous  
system. They include, but are not limited to, neuropeptides,  
25 amino acids, biogenic amines, lipids and lipid metabolites,  
and other metabolic byproducts. Many of these neuroregulator  
substances interact with specific cell surface receptors  
which transduce signals from the outside to the inside of the  
cell. G-protein coupled receptors (GPCRs) represent a major  
30 class of cell surface receptors with which many  
neurotransmitters interact to mediate their effects. GPCRs  
are characterized by seven membrane-spanning domains and are  
coupled to their effectors via G-proteins linking receptor  
activation with intracellular biochemical sequelae such as  
35 stimulation of adenylyl cyclase.

Opsins represent one of the major families of GPCRs. These

Rhodopsin contains the seven membrane-spanning apoprotein opsin and a retinoid-based chromophore (See reviews Hargrave and McDowell, 1992; Yarfitz and Hurley, 1994). In the ground or inactive state (i.e. in the absence of light), the chromophore, usually 11-*cis*-retinal, is covalently attached to a highly conserved lysine residue in the middle of the seventh transmembrane segment via a protonated Schiff base. All vertebrate visual opsins contain a highly conserved glutamate residue in the transmembrane helix 3 which serves



-3-

as a counterion for the protonated Schiff base. It has been postulated that 11-cis-retinal behaves as an inverse agonist and induces an inactive conformation of the apoprotein which, by itself, is partially active (Cohen et al., 1993; Surya et al., 1995). Upon absorbing a photon, 11-cis-retinal is isomerized to the agonist all-trans-retinal which introduces distortion in the opsin and initiates a cascade of conformational changes in the molecule. Rhodopsin is first converted to bathorhodopsin, followed by lumirhodopsin, metarhodopsin I and metarhodopsin II states in a sequential manner. Even though most of these transient conformational states are difficult to study biochemically, they can be easily distinguished on the basis of their spectroscopic properties since each state has a unique absorption maximum. Experimental evidence suggests that the formation of metarhodopsin II, a relatively stable state, involves deprotonation of the Schiff base and represents the active conformation of the apoprotein. In this state, the opsin activates the cognate G-protein and initiates the intracellular signaling cascade which ultimately results in transfer of visual information to the brain. Upon hydrolysis of the Schiff base linkage, metarhodopsin II decays into free all-trans-retinal and opsin. All-trans-retinal is transported to the neighbouring retinal pigment epithelial cells where it is converted to 11-cis-retinal via enzymatic reactions. 11-cis-retinal is transported back to retinal photoreceptors where it recombines with the opsin apoprotein to regenerate the rhodopsin molecule.

Even though all visual opsins essentially use the same activation mechanism as rhodopsin, there are some noticeable differences between vertebrate and invertebrate visual opsins (Gartner and Towner, 1995; Yarfitz and Hurley, 1994, Terakita et al., 1998; Arnheiter, 1998). Activation of vertebrate visual pigment results primarily in stimulation of G<sub>t</sub> G-

-4-

protein (also known as transducin) leading to an increase in cGMP phosphodiesterase activity. Initiation of this signaling cascade ultimately results in closure of cation channels and hyperpolarization of the cell. In contrast, opsin visual pigments in invertebrates such as squid and fruitfly activate  $G_q$  G-protein and elevate intracellular  $IP_3$  and  $Ca^{2+}$  levels (Wood et al., 1989; Nobes et al., 1992; Yarfitz and Hurley, 1994 ). Another major difference between vertebrate and invertebrate visual opsins is the stability of the active conformation of the receptor. Formation of vertebrate metarhodopsin II, the active conformation of rhodopsin, is rapidly followed by hydrolysis of the Schiff base linkage and dissociation of metarhodopsin II into free all-trans-retinal and opsin apoprotein. It has been suggested that the glutamate counterion in the transmembrane helix 3 aids in the hydrolysis reaction (Gartner and Towner, 1995). In contrast, invertebrate metarhodopsin represents a thermally stable state where the chromophore remains attached to the apoprotein (Kiselev and Subramaniam, 1994). This allows rapid photoisomerization of all-trans-retinal back to 11-cis-retinal within the apoprotein and rapid regeneration of rhodopsin, thus eliminating the need for retinal regenerating tissue (Provencio et al., 1998). The thermally stable metastate of invertebrate photopigment may be formed due to the absence of the glutamate counterion in transmembrane helix 3 of invertebrate visual opsins (Gartner and Towner, 1995).

Most opsins use 11-cis-retinal derived from carotenoids as a chromophore; however, some opsins use 3-hydroxy, 4-hydroxy or 3,4-dehydro isomers of 11-cis-retinal as a chromophore to accommodate the abundant availability of the substituted carotenoids (Gartner and Towner, 1995). Different opsins respond to photons with different wavelengths, a phenomenon known as spectral tuning. Even though the use of a

-5-

particular retinal derivative as a chromophore contributes to spectral specificity (Gartner and Towner, 1995), the major determinant of spectral tuning is the presence of unique amino acids surrounding the retinal-binding site (Kochendoerfer et al., 1999). For example, substitution of a highly conserved glycine in transmembrane helix 3 of rhodopsin with amino acids of increasing size results in progressive shift of  $\lambda_{\max}$  towards the blue wavelength (Han et al., 1996). Similarly, replacement of conserved non-polar residues with hydroxyl amino acids changes the opsin from a green-absorbing molecule to a red-absorbing pigment (Chan et al., 1992).

Even though the visual opsins have been at the forefront of opsin research, scientists are now turning their attention to non-visual opsins (the opsins not involved in transducing visual information) because of their potential involvement in physiological processes such as circadian rhythm and reproduction. The existence of non-visual photopigments in nonmammalian vertebrates was first suggested by Karl von Frisch. He demonstrated that the skin of the European minnow changed color in response to light even in the absence of the eye and pineal gland, and postulated photoreceptive elements in the diencephalon (Foster et al, 1994). Further evidence supporting the presence of non-visual photopigments was obtained in blinded lampreys and ducks which responded to illumination with body movements and gonadal induction, respectively (Foster et al., 1994). Recent studies using histochemical techniques has further corroborated these physiological observations. Silver et al. (1988) immunostained the cerebrospinal fluid (CSF)-contacting neurons with anti-opsin antibody in brains of the ring dove, quail and duck. Similarly, intense immunostaining of the CSF-contacting neurons within the basal brain of the lizard, *Anolis carolinensis*, was observed with anti-cone opsin

-6-

antibody (Foster et al., 1993).

Recent molecular cloning of several non-visual opsins is in agreement with the above-mentioned studies. Pinopsin is expressed in the pineal gland of the chicken and is believed to play a role in circadian rhythm (Okano et al., 1994; Max et al., 1995). Interestingly, expression of the pinopsin gene is regulated by light (Takanaka et al., 1998). Max et al. (1998) have demonstrated light-dependent activation of transducin by pinopsin, implying that the pinopsin is a functional photoreceptor. Two other opsins identified in pinealocytes are vertebrate ancient (VA) opsin cloned from the salmon fish (Soni et al., 1997; Soni et al., 1998), and parapinopsin cloned from the channel catfish (Blackshaw and Snyder, 1997). In addition to pineal cells, VA opsin is also localized in the amacrine and horizontal cells of the salmon retina. On the other hand, expression of parapinopsin is confined to the parapineal and pineal organs of the catfish.

Several of the non-visual opsins are, in fact, expressed in the eye. Sun et al. (1997) cloned peropsin from human retina and mouse eye. This opsin is localized exclusively in microvilli of the apical membrane of retinal pigment epithelial (RPE) cells, indicating that it may function as a sensor of retinoids generated in the adjacent outer membrane of rhodopsin or cone opsins. RPE retinal G-protein-coupled receptor (RGR) is another receptor found in the RPE (Tao et al., 1998). Unlike other opsins which are believed to be present at the plasma membrane, RGR is localized intracellularly. The amino acid sequence of RGR suggests that, along with squid retinochrome, it may form a distinct subfamily of opsins (Hara-Nishimura et al., 1990).

Interestingly, it has been suggested that RGR may prefer all-*trans*-retinal, rather than 11-*cis*-retinal, as a ligand and

-7-

may be involved in the photoisomerization of the all-trans isomer to the 11-cis isomer (Hao and Fong, 1999). In such a case, its function may be the rapid regeneration of 11-cis-retinal in the RPE for use in the visual cycle.

5

One of the known photo-sensitive processes is melanosome dispersion in the dermal melanophores of *Xenopus laevis*. In accordance with this, melanopsin has been cloned from melanophores (Provencio et al., 1998). Melanopsin is expressed in the melanophores, suprachiasmatic and preoptic nuclei of the hypothalamus, iris, RPE and retina. Its expression in visual and nonvisual tissues suggests a role in visual and nonvisual photosensory phenomena. Recently, a non-visual opsin has been identified in the mammalian brain. Blackshaw and Snyder (1999) have cloned encephalopsin, which, as the name suggests, is highly expressed in various areas of the brain. It is present in the preoptic area and the paraventricular nucleus of the hypothalamus, the cerebral cortex, cerebellar Purkinje cells, striatum, thalamus and the ventral horn of the spinal cord. Interestingly, this receptor is not present in the eye.

10

15

20

25

30

35

The molecular identification of non-visual opsins has raised several questions. How are they activated? What is their physiological function? All the non-visual opsins cloned to date contain lysine in the seventh transmembrane helix, the site for retinal chromophore attachment, implying that a retinoid may be the chromophore for the non-visual opsins, similar to the visual opsins. Several groups have been successful in reconstituting non-visual opsins with retinoids and activating them with light (Okano et al., 1994; Soni et al., 1998). Retinoids can cross the blood-brain barrier, albeit at low efficiency (Pardridge et al., 1985; Franke et al., 1999). Furthermore, a transporter with high affinity for retinoids,  $\beta$ -Trace, has been recognized recently (Tanaka

-8-

et al., 1997). This secretory protein is present in high levels in the CSF and may transport retinoids to different regions of the brain, analogous to the plasma RBP. That retinoids are indeed present in the brain was demonstrated by Foster et al. (1993) who were able to identify retinal isomers in the *Anolis* anterior brain using HPLC analysis. If a retinal isomer is indeed a chromophore for non-visual opsins then light would be needed to photoisomerize the isomer and activate the receptor. Several reports suggest that light can reach the deep areas of the brain (Muller and Wilson, 1986; Grace et al., 1996; Blackshaw and Snyder, 1999), and a neurotransmitter release-enhancing effect of light on cortical slices has been observed (Wade et al., 1988). Therefore, the activation mechanisms of non-visual opsins may be similar to the visual opsins. However, it should be noted here that some non-visual opsins have proven resistant to functional reconstitution with retinal isomers (Provencio et al., 1998; Blackshaw and Snyder, 1999), raising the possibility that these receptors utilize a non-retinoid ligand and may not require light for activation.

What could be the function of non-visual opsins? One interesting possibility is that they may be involved in circadian rhythm. Circadian rhythm represents daily fluctuations in biological activities that are regulated by the light-dark cycles. It is composed of three components: a photoactive input, the circadian clock itself which exhibits periodicity, and the behavioral and physiological oscillations as output. In animals, in the phenomenon known as photoentrainment, exposure to light results in regulation of the circadian rhythm. However, the identity of the photoreceptive molecule mediating photoentrainment has remained a mystery. Since the photoentrainment response occurs at the wavelength of 500 nm, it has been suggested that an opsin may be mediating the response (Foster, 1998).

-9-

Freedman et al. (1999) and Lucas et al. (1999) recently demonstrated that photoentrainment was intact in mice lacking rod and cone receptors; however, removal of the eyes in these mice abolished the effect of light on circadian rhythm as well as on melatonin synthesis. These results, and ocular localization of several non-visual opsins, strongly support the role of ocular non-visual opsins in photoentraining circadian rhythm. In addition, the non-visual opsins localized in the CNS may form a component of the circadian clock itself.

If non-visual opsins are indeed involved in regulating circadian rhythm, then they represent an attractive therapeutic target for circadian rhythm-related conditions. These include sleep disorders such as jet lag. It has been suggested that a change in circadian rhythm may be an underlying cause for sleep disorders such as insomnia, Advanced Sleep Phase Syndrome and Delayed Sleep Phase Syndrome (Sedgwick, 1998; Refinetti, 1999). In addition, dissociation between biological clock and work hours may result in shift-work-related sleep disorders. Importantly, bright light therapy has been demonstrated to help in these disorders (Rosenthal et al., 1990; Lack and Wright, 1993; Campbell et al., 1995; Murphy and Campbell, 1996; Cooke et al., 1998; Refinetti, 1999). Similarly, exposure to light at appropriate time reduces the effect of jet lag on travelers (Refinetti, 1999). These observations suggest that non-visual opsins may mediate these beneficial effects of light in circadian rhythm-related disorders.

Non-visual opsins also may play a role in seasonal affective disorder (SAD). This disorder is characterized by a subpopulation of people suffering from depression during winter. Light therapy is effective in these people (Terman and Terman, 1999); especially green light is more effective than red light (Oren et al., 1991). It has been recently

-10-

hypothesized that the interaction of specialized photoreceptors with magnetic field may influence sensitivity of patients suffering from SAD to light (Partonen, 1998).

5 In addition, the discreet localization of various opsins in the CNS areas indicate their potential role in CNS-related physiology and disorders.

10 Non-visual photoreceptors are also involved in melanosome dispersion in melanophores, and thus, in change in the color of the skin in various species. In birds and mammals, non-visual 'deep brain photoreceptors' are also linked to reproductive behaviour and photoperiodic gonadal responses (Yoshikawa and Oishi, 1998).

15 In summary, opsins constitute an important branch of the GPCR superfamily. They behave as photosensitive elements. They are localized in the retina and in non-retinal locations including the brain. The retinal rod and cone opsins are  
20 mainly responsible for conveying visual information to the brain, while the non-visual opsins in the retina and elsewhere may be involved in regulation of melatonin synthesis and circadian rhythm, photoentrainment, SAD, skin colour change and camouflage, and reproductive behaviour.



-11-

SUMMARY OF THE INVENTION

This invention provides an isolated nucleic acid encoding a mammalian SNORF36 receptor.

5

This invention further provides a purified mammalian SNORF36 receptor protein.

This invention also provides a vector comprising a nucleic acid in accordance with this invention.

10

This invention still further provides a cell comprising a vector in accordance with this invention.

This invention additionally provides a membrane preparation isolated from a cell in accordance with this invention.

15

Furthermore, this invention provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within one of the two strands of the nucleic acid encoding the mammalian SNORF36 receptor contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977), plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976), plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534) or plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

20

25

This invention further provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO: 1), (b) the

30

35

5

10

15

20

25

30

35

-13-

This invention also provides a pharmaceutical composition which comprises an amount of an antibody in accordance with this invention effective to block binding of a ligand to a human SNORF36 receptor and a pharmaceutically acceptable carrier.

This invention further provides a transgenic, nonhuman mammal expressing DNA encoding a mammalian SNORF36 receptor in accordance with this invention.

This invention still further provides a transgenic, nonhuman mammal comprising a homologous recombination knockout of a native mammalian SNORF36 receptor.

This invention further provides a transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a mammalian SNORF36 receptor in accordance with this invention so placed within such genome as to be transcribed into antisense mRNA which is complementary to and hybridizes with mRNA encoding the mammalian SNORF36 receptor so as to reduce translation of such mRNA and expression of such receptor.

This invention provides a process for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises contacting cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor.

This invention further provides a process for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises contacting a membrane

-14-

preparation from cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor.

This invention still further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises separately contacting cells expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF36 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF36 receptor.

This invention further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises separately contacting a membrane preparation from cells expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the

-15-

mammalian SNORF36 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF36 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF36 receptor.

This invention further provides a compound identified by one of the processes of this invention.

This invention provides a method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF36 receptor to identify a compound which specifically binds to the mammalian SNORF36 receptor, which comprises (a) contacting cells transfected with, and expressing, DNA encoding the mammalian SNORF36 receptor with a compound known to bind specifically to the mammalian SNORF36 receptor; (b) contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian SNORF36 receptor, under conditions permitting binding of compounds known to bind to the mammalian SNORF36 receptor; (c) determining whether the binding of the compound known to bind to the mammalian SNORF36 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the mammalian SNORF36 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF36 receptor.

This invention further provides a method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF36 receptor to identify a compound which specifically binds to the mammalian SNORF36 receptor, which comprises (a) contacting a membrane preparation from cells transfected with, and expressing, DNA encoding the mammalian

-16-

SNORF36 receptor with the plurality of compounds not known to bind specifically to the mammalian SNORF36 receptor under conditions permitting binding of compounds known to bind to the mammalian SNORF36 receptor; (b) determining whether the binding of a compound known to bind to the mammalian SNORF36 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (c) separately determining the binding to the mammalian SNORF36 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF36 receptor.

This invention also provides a method of detecting expression of a mammalian SNORF36 receptor by detecting the presence of mRNA coding for the mammalian SNORF36 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with a nucleic acid probe according to this invention under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the mammalian SNORF36 receptor by the cell.

This invention further provides a method of detecting the presence of a mammalian SNORF36 receptor on the surface of a cell which comprises contacting the cell with an antibody according to this invention under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of the mammalian SNORF36 receptor on the surface of the cell.

This invention still further provides a method of determining the physiological effects of varying levels of activity of mammalian SNORF36 receptors which comprises producing a

-17-

transgenic, nonhuman mammal in accordance with this invention whose levels of mammalian SNORF36 receptor activity are varied by use of an inducible promoter which regulates mammalian SNORF36 receptor expression.

5

This invention additionally provides a method of determining the physiological effects of varying levels of activity of mammalian SNORF36 receptors which comprises producing a panel of transgenic, nonhuman mammals in accordance with this invention each expressing a different amount of mammalian SNORF36 receptor.

10

Moreover, this invention provides a method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor comprising administering a compound to a transgenic, nonhuman mammal according to this invention, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal as a result of overactivity of a mammalian SNORF36 receptor, the alleviation of such an abnormality identifying the compound as an antagonist.

15

20

This invention also provides an antagonist identified by the preceding method.

25

This invention further provides a composition, e.g. a pharmaceutical composition, comprising an antagonist according to this invention and a carrier, e.g. a pharmaceutically acceptable carrier.

30

This invention additionally provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition according

35

-18-

to this invention so as to thereby treat the abnormality.

In addition, this invention provides a method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor comprising administering a compound to a transgenic, nonhuman mammal according to this invention, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal, the alleviation of such an abnormality identifying the compound as an agonist.

This invention further provides an agonist identified by the preceding method.

This invention still further provides a composition, e.g. a pharmaceutical composition, comprising an agonist according to this invention and a carrier, e.g. a pharmaceutically acceptable carrier.

Moreover, this invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition according to this invention so as to thereby treat the abnormality.

Yet further, this invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific mammalian allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b) performing a restriction digest of the DNA with a panel of restriction enzymes; (c) electrophoretically separating the resulting DNA fragments on a sizing gel; (d) contacting the resulting gel with a nucleic acid probe capable of



-19-

specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian SNORF36 receptor and labeled with a detectable marker; (e) detecting labeled bands which have hybridized to the DNA encoding a mammalian SNORF36 receptor to create a unique band pattern specific to the DNA of subjects suffering from the disorder; (f) repeating steps (a)-(e) with DNA obtained for diagnosis from subjects not yet suffering from the disorder; and (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) with the band pattern from step (f) for subjects not yet suffering from the disorder so as to determine whether the patterns are the same or different and thereby diagnose predisposition to the disorder if the patterns are the same.

This invention also provides a method of preparing a purified mammalian SNORF36 receptor according to the invention which comprises: (a) culturing cells which express the mammalian SNORF36 receptor; (b) recovering the mammalian SNORF36 receptor from the cells; and (c) purifying the mammalian SNORF36 receptor so recovered.

This invention further provides a method of preparing the purified mammalian SNORF36 receptor according to the invention which comprises: (a) inserting a nucleic acid encoding the mammalian SNORF36 receptor into a suitable expression vector; (b) introducing the resulting vector into a suitable host cell; (c) placing the resulting host cell in suitable conditions permitting the production of the mammalian SNORF36 receptor; (d) recovering the mammalian SNORF36 receptor so produced; and optionally (e) isolating and/or purifying the mammalian SNORF36 receptor so recovered.

Furthermore, this invention provides a process for determining whether a chemical compound is a mammalian

-20-

SNORF36 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF36 receptor with the compound under conditions permitting the activation of the mammalian SNORF36 receptor, and detecting any increase in mammalian SNORF36 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF36 receptor agonist.

This invention also provides a process for determining whether a chemical compound is a mammalian SNORF36 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF36 receptor with the compound in the presence of a known mammalian SNORF36 receptor agonist, under conditions permitting the activation of the mammalian SNORF36 receptor, and detecting any decrease in mammalian SNORF36 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF36 receptor antagonist.

This invention still further provides a composition, for example a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor agonist determined by a process according to this invention effective to increase activity of a mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF36 receptor agonist is not previously known.

Also, this invention provides a composition, for example a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor antagonist determined by a process according to this invention effective to reduce activity of a mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier.

This invention moreover provides a process for determining

-21-

whether a chemical compound specifically binds to and activates a mammalian SNORF36 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the chemical compound under conditions suitable for activation of the mammalian SNORF36 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change, e.g. an increase, in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian SNORF36 receptor.

This invention still further provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian SNORF36 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to activate the mammalian SNORF36 receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian SNORF36 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change, e.g. increase, in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian SNORF36 receptor.

Further, this invention provides a compound determined by a process according to the invention and a composition, for

-22-

example, a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor agonist determined to be such by a process according to the invention, effective to increase activity of the mammalian SNORF36 receptor and  
5 a carrier, for example, a pharmaceutically acceptable carrier.

This invention also provides a composition, for example, a pharmaceutical composition, which comprises an amount of a  
10 mammalian SNORF36 receptor antagonist determined to be such by a process according to the invention, effective to reduce activity of the mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier.

15 This invention yet further provides a method of screening a plurality of chemical compounds not known to activate a mammalian SNORF36 receptor to identify a compound which activates the mammalian SNORF36 receptor which comprises:  
(a) contacting cells transfected with and expressing the  
20 mammalian SNORF36 receptor with the plurality of compounds not known to activate the mammalian SNORF36 receptor, under conditions permitting activation of the mammalian SNORF36 receptor; (b) determining whether the activity of the mammalian SNORF36 receptor is increased in the presence of  
25 one or more of the compounds; and if so (c) separately determining whether the activation of the mammalian SNORF36 receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian SNORF36 receptor.

30 This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian SNORF36 receptor to identify a compound which inhibits the activation of the mammalian SNORF36 receptor,  
35 which comprises: (a) contacting cells transfected with and expressing the mammalian SNORF36 receptor with the plurality

-23-

of compounds in the presence of a known mammalian SNORF36 receptor agonist, under conditions permitting activation of the mammalian SNORF36 receptor; (b) determining whether the extent or amount of activation of the mammalian SNORF36 receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian SNORF36 receptor in the absence of such one or more compounds; and if so (c) separately determining whether each such compound inhibits activation of the mammalian SNORF36 receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of compounds which inhibits the activation of the mammalian SNORF36 receptor.

This invention also provides a composition, for example a pharmaceutical composition, comprising a compound identified by a method according to this invention in an amount effective to increase mammalian SNORF36 receptor activity and a carrier, for example, a pharmaceutically acceptable carrier.

This invention still further provides a composition, for example a pharmaceutical composition, comprising a compound identified by a method according to this invention in an amount effective to decrease mammalian SNORF36 receptor activity and a carrier, for example a pharmaceutically acceptable carrier.

Furthermore, this invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject a compound which is a mammalian SNORF36 receptor agonist in an amount effective to treat the abnormality.

This invention additionally provides a method of treating an

-24-

abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject a compound which is a mammalian SNORF36 receptor antagonist in an amount effective to treat the abnormality.

This invention also provides a process for making a composition of matter which specifically binds to a mammalian SNORF36 receptor which comprises identifying a chemical compound using a process in accordance with this invention and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

This invention further provides a process for preparing a composition, for example, a pharmaceutical composition which comprises admixing a carrier, for example, a pharmaceutically acceptable carrier, and a pharmaceutically effective amount of a chemical compound identified by a process of in accordance with this invention or a novel structural and functional analog or homolog thereof.

**BRIEF DESCRIPTION OF THE FIGURES****Figures 1A-1C**

5 Nucleotide sequence including sequence encoding a human  
SNORF36a receptor (SEQ ID NO: 1). Putative open reading  
frames including the shortest open reading frame are  
indicated by underlining two start (ATG) codons (at positions  
10-12 and 67-69) and the stop codon (at positions 1444-1446).  
10 In addition, partial 5' and 3' untranslated sequences are  
shown.

**Figures 2A-2B**

15 Deduced amino acid sequence (SEQ ID NO: 2) of the human  
SNORF36a receptor encoded by the longest open reading frame  
indicated in the nucleotide sequence shown in Figures 1A-1C  
(SEQ ID NO: 1). The seven putative transmembrane (TM) regions  
are underlined.

**Figures 3A-3C**

20 Nucleotide sequence including sequence encoding a human  
SNORF36b receptor (SEQ ID NO: 3). Putative open reading  
frames including the shortest open reading frame are  
indicated by underlining two start (ATG) codons (at positions  
10-12 and 67-69) and the stop codon (at positions 1477-1479).  
25 In addition, partial 5' and 3' untranslated sequences are  
shown.

**Figures 4A-4C**

30 Deduced amino acid sequence (SEQ ID NO: 4) of the human  
SNORF36b receptor encoded by the longest open reading frame  
indicated in the nucleotide sequence shown in Figures 3A-3C  
(SEQ ID NO: 3). The seven putative transmembrane (TM) regions  
are underlined.

**Figure 5**

Nucleotide sequence including part of the sequence encoding a rat SNORF36 receptor (SEQ ID NO: 5).

**Figure 6**

Deduced partial amino acid sequence (SEQ ID NO: 6) of the rat SNORF36 receptor encoded by the nucleotide sequence shown in Figure 5 (SEQ ID NO: 5). Putative transmembrane (TM) regions are underlined.

**Figures 7A-7B**

Nucleotide alignment of partial sequences of human SNORF36 and rat SNORF36. Vertical lines represent identical residues.

**Figure 8**

Amino acid alignment of partial sequences of human SNORF36 and rat SNORF36. Vertical lines represent identical residues and dots represent similar residues.

**Figures 9A-9C**

Nucleotide sequence including sequence encoding a rat SNORF36 receptor (SEQ ID NO: 7). Putative open reading frames including the shortest open reading frame are indicated by underlining one start (ATG) codon (at positions 25-27) and the stop codon (at positions 1447-1449). In addition, partial 5' and 3' untranslated sequences are shown.

**Figures 10A-10C**

Deduced amino acid sequence (SEQ ID NO: 8) of the rat SNORF36 receptor encoded by the longest open reading frame indicated in the nucleotide sequence shown in Figures 9A-9C (SEQ ID NO: 7). The seven putative transmembrane (TM) regions are underlined.



-27-

**Figures 11A-11D**

Nucleotide alignment of human and rat SNORF36 receptors. Vertical lines indicate conserved residues, dots represent gaps in the alignment.

**Figures 12A-12B**

Amino acid alignment of human and rat SNORF36 receptors. Vertical lines indicate conserved residues, dots represent gaps in the alignment.

**Figures 13A and 13B**

Baseline  $\text{Ca}^{2+}$  mobilization response in human SNORF36a-transfected Cos-7 cells which were either, (1) not exposed to lamp light (Figure 13A), or (2) exposed to lamp light for 90 minutes before the experiment (Figure 13B). The response was measured using Fluorometric Imaging Plate Reader (FLIPR™). Cells were exposed to laser light at 1 second interval beginning at time 0 until the end of the trace. The first fluorescence value was subtracted from all the fluorescence values. No drug was added during the duration of the trace.

**Figures 14A and 14B**

Representative traces demonstrating the effect of all-trans-retinal (10 $\mu\text{M}$ ) on intracellular  $\text{Ca}^{2+}$  in (1) human SNORF36a-transfected (Figure 14A), and (2) empty vector-transfected Cos-7 cells (Figure 14B). The response was measured using Fluorometric Imaging Plate Reader (FLIPR™). All-trans-retinal was added at time indicated by the arrow. Baseline subtraction and negative control corrections were performed on the traces.

**Figures 15A and 15B**

Concentration-dependent effect of retinal analogues on intracellular  $\text{Ca}^{2+}$  in human SNORF36a-transfected (1) Cos-7

-28-

cells (n=3) (Figure 15A), and (2) HEK293 cells (n=1) (Figure 15B). Data are presented as mean  $\pm$  SD.

**Figures 16A and 16B**

Concentration-dependent effect of all-trans-retinal on phosphoinositide hydrolysis in human SNORF36a- and empty vector-transfected Cos-7 cells either (1) exposed to lamp light (n=2) (Figure 16A), or (2) not exposed to lamp light (n=1) (Figure 16B). Data are presented as mean  $\pm$  SD.

**Figures 17A and 17B**

Antagonism by  $\beta$ -ionone (10 $\mu$ M) of all-trans-retinal-induced intracellular Ca<sup>2+</sup> mobilization in human SNORF36a-transfected Cos-7 cells (n=2) (Figure 17A). Antagonism by  $\beta$ -ionone (10 $\mu$ M) of 9-cis-retinal-induced intracellular Ca<sup>2+</sup> mobilization in human SNORF36a-transfected Cos-7 cells (n=2) (Figure 17B). The response was measured using Fluorometric Imaging Plate Reader (FLIPR™). Data are presented as mean  $\pm$  SD.

**Figures 18A-18C**

Light sensitivity of SNORF36a expressed in oocytes. Oocytes shown in Figure 18A and Figure 18B were injected with 10 ng SNORF36a synthetic mRNA. The current is expressed in nA (nanoampere) while time is expressed in s (second).

**Figure 18A:** Current elicited by light exposure (bar) in a voltage-clamped oocyte expressing SNORF36a. The oocyte was pre-incubated with all-trans-retinal (100 nM) in the dark for 24 hours.

**Figure 18B:** Response of a second oocyte pre-incubated with all-trans-retinal that had been previously exposed to room light.

**Figure 18C:** Control uninjected oocyte, pre-incubated with

-29-

all-trans-retinal (100 nM) for 24 h, fails to respond to light.

**Figure 19**

- 5 Summary of light sensitivity of oocytes expressing SNORF36 pre-incubated with all-trans-retinal, 13-cis-retinal and ATRA (all-trans retinoic acid). Numbers in parentheses are the numbers of oocytes used.

**DETAILED DESCRIPTION OF THE INVENTION**

This invention provides a recombinant nucleic acid comprising a nucleic acid encoding a mammalian SNORF36a receptor, wherein the mammalian receptor-encoding nucleic acid hybridizes under high stringency conditions to a nucleic acid encoding a human SNORF36a receptor and having a sequence identical to the sequence of the human SNORF36a receptor-encoding nucleic acid contained in plasmid pCDNA3.1-hSNORF36a-f (ATCC Patent Depository No. 203977).

This invention further provides a recombinant nucleic acid comprising a nucleic acid encoding a human SNORF36a receptor, wherein the human SNORF36a receptor comprises an amino acid sequence identical to the sequence of the human SNORF36a receptor encoded by the shortest open reading frame indicated in Figures 1A-1C (SEQ ID NO: 1).

This invention provides a recombinant nucleic acid comprising a nucleic acid encoding a mammalian SNORF36b receptor, wherein the mammalian receptor-encoding nucleic acid hybridizes under high stringency conditions to a nucleic acid encoding a human SNORF36b receptor and having a sequence identical to the sequence of the human SNORF36b receptor-encoding nucleic acid contained in plasmid pCDNA3.1-hSNORF36b-f (ATCC Patent Depository No. 203976).

This invention also provides a recombinant nucleic acid comprising a nucleic acid encoding a human SNORF36b receptor, wherein the human SNORF36b receptor comprises an amino acid sequence identical to the sequence of the human SNORF36b receptor encoded by the shortest open reading frame indicated in Figures 3A-3C (SEQ ID NO: 3).

This invention also contemplates recombinant nucleic acids

-31-

which comprise nucleic acids encoding naturally occurring allelic variants of the above. For example, one such allelic variant involves changing Guanine (G) to Adenine (A) at position 39 in Figures 1A-1C (SEQ ID NO: 1). Another example of such an allelic variant involves changing Guanine (G) to Adenine (A) at position 39 in Figures 3A-3C (SEQ ID NO: 3).

The plasmid pcDNA3.1-hSNORF36a-f and plasmid pcDNA3.1-hSNORF36b-f were both deposited on April 28, 1999, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Patent Depository Nos. 203977 and 203976, respectively.

The plasmid pEXJ.T3T7-rSNORF36p was deposited on August 17, 1999, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Patent Depository No. PTA-534.

The plasmid pEXJ.T7-rSNORF36-f was deposited on January 18, 2000, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Patent Depository No. PTA-1216.

Hybridization methods are well known to those of skill in the art. For purposes of this invention, hybridization under high stringency conditions means hybridization performed at 40°C in a hybridization buffer containing 50% formamide, 5X SSC,

-32-

7mM Tris, 1X Denhardt's, 25µg/ml salmon sperm DNA; wash at 50°C in 0.1X SSC, 0.1%SDS.

Throughout this application, the following standard abbreviations are used to indicate specific nucleotide bases:

A = adenine

G = guanine

C = cytosine

T = thymine

10 M = adenine or cytosine

R = adenine or guanine

W = adenine or thymine

S = cytosine or guanine

Y = cytosine or thymine

15 K = guanine or thymine

V = adenine, cytosine, or guanine (not thymine)

H = adenine, cytosine, or thymine (not cytosine)

B = cytosine, guanine, or thymine (not adenine)

20 N = adenine, cytosine, guanine, or thymine (or other modified base such as inosine)

I = inosine

Furthermore, the term "agonist" is used throughout this application to indicate any peptide or non-peptidyl compound which increases the activity of any of the polypeptides of the subject invention. The term "antagonist" is used throughout this application to indicate any peptide or non-peptidyl compound which decreases the activity of any of the polypeptides of the subject invention.

30 Furthermore, as used herein, the phrase "pharmaceutically acceptable carrier" means any of the standard pharmaceutically acceptable carriers. Examples include, but are not limited to, phosphate buffered saline, physiological saline, water, and emulsions, such as oil/water emulsions.

-33-

It is possible that the mammalian SNORF36 receptor gene contains introns and furthermore, the possibility exists that additional introns could exist in coding or non-coding regions. In addition, spliced form(s) of mRNA may encode additional amino acids either upstream of the currently defined starting methionine or within the coding region. Further, the existence and use of alternative exons is possible, whereby the mRNA may encode different amino acids within the region comprising the exon. In addition, single amino acid substitutions may arise via the mechanism of RNA editing such that the amino acid sequence of the expressed protein is different than that encoded by the original gene. (Burns, et al., 1996; Chu, et al., 1996). Such variants may exhibit pharmacologic properties differing from the polypeptide encoded by the original gene.

This invention provides splice variants of the mammalian SNORF36 receptors disclosed herein. This invention further provides for alternate translation initiation sites and alternately spliced or edited variants of nucleic acids encoding the SNORF36 receptors of this invention.

This invention also contemplates recombinant nucleic acids which comprise nucleic acids encoding naturally occurring allelic variants of the SNORF36 receptors disclosed herein.

The nucleic acids of the subject invention also include nucleic acid analogs of the human SNORF36a receptor genes, wherein the human SNORF36a receptor gene comprises the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO: 1) or contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). Nucleic acid analogs of the human SNORF36a receptor genes differ from the human SNORF36a receptor genes described herein in terms of the identity or location of one or more nucleic acid bases (deletion analogs containing less than all of the nucleic acid bases shown in Figures 1A-1C or

The nucleic acids of the subject invention also include nucleic acid analogs of the human SNORF36b receptor genes, wherein the human SNORF36b receptor gene comprises the nucleic acid sequence shown in Figures 3A-3C (SEQ ID NO: 3)



or contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). Nucleic acid analogs of the human SNORF36b receptor genes differ from the human SNORF36b receptor genes described herein in terms of the identity or location of one or more nucleic acid bases (deletion analogs containing less than all of the nucleic acid bases shown in Figures 3A-3C or contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976), substitution analogs wherein one or more nucleic acid bases shown in Figures 3A-3C or contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976), are replaced by other nucleic acid bases, and addition analogs, wherein one or more nucleic acid bases are added to a terminal or medial portion of the nucleic acid sequence) and which encode proteins which share some or all of the properties of the proteins encoded by the nucleic acid sequences shown in Figures 3A-3C or contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In one embodiment of the present invention, the nucleic acid analog encodes a protein which has an amino acid sequence identical to that shown in Figures 4A-4C or encoded by the nucleic acid sequence contained in pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In another embodiment, the nucleic acid analog encodes a protein having an amino acid sequence which differs from the amino acid sequences shown in Figures 4A-4C or encoded by the nucleic acid contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In a further embodiment, the protein encoded by the nucleic acid analog has a function which is the same as the function of the receptor proteins having the amino acid sequence shown in Figures 4A-4C. In another embodiment, the function of the protein encoded by the nucleic acid analog differs from the function of the receptor protein having the amino acid sequence shown in Figures 4A-4C. In another embodiment, the variation in the nucleic acid sequence occurs within the transmembrane (TM) region of the protein. In a further embodiment, the variation in the nucleic acid sequence occurs

-36-

outside of the TM region.

The nucleic acids of the subject invention also include nucleic acid analogs of the rat SNORF36 receptor genes, wherein the rat SNORF36 receptor gene comprises the nucleic acid sequence shown in Figure 5 or contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). Nucleic acid analogs of the rat SNORF36 receptor genes differ from the rat SNORF36 receptor genes described herein in terms of the identity or location of one or more nucleic acid bases deletion analogs containing less than all of the nucleic acid bases shown in Figure 5 or contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534), substitution analogs wherein one or more nucleic acid bases shown in Figure 5 or contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534), are replaced by other nucleic acid bases, and addition analogs, wherein one or more nucleic acid bases are added to a terminal or medial portion of the nucleic acid sequence) and which encode proteins which share some or all of the properties of the proteins encoded by the nucleic acid sequences shown in Figure 5 or contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). In one embodiment of the present invention, the nucleic acid analog encodes a protein which has an amino acid sequence identical to that shown in Figure 6 or encoded by the nucleic acid sequence contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). In another embodiment, the nucleic acid analog encodes a protein having an amino acid sequence which differs from the amino acid sequences shown in Figure 6 or encoded by the nucleic acid contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). In a further embodiment, the protein encoded by the nucleic acid analog has a function which is the same as the function of the receptor proteins having the amino acid sequence shown in Figure 6. In another embodiment, the function of the protein encoded by the

-37-

nucleic acid analog differs from the function of the receptor protein having the amino acid sequence shown in Figure 6. In another embodiment, the variation in the nucleic acid sequence occurs within the transmembrane (TM) region of the protein. In a further embodiment, the variation in the nucleic acid sequence occurs outside of the TM region.

The nucleic acids of the subject invention also include nucleic acid analogs of the rat SNORF36 receptor genes, wherein the rat SNORF36 receptor gene comprises the nucleic acid sequence shown in Figures 9A-9C or contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). Nucleic acid analogs of the rat SNORF36 receptor genes differ from the rat SNORF36 receptor genes described herein in terms of the identity or location of one or more nucleic acid bases deletion analogs containing less than all of the nucleic acid bases shown in Figures 9A-9C or contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216), substitution analogs wherein one or more nucleic acid bases shown in Figures 9A-9C or contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216), are replaced by other nucleic acid bases, and addition analogs, wherein one or more nucleic acid bases are added to a terminal or medial portion of the nucleic acid sequence) and which encode proteins which share some or all of the properties of the proteins encoded by the nucleic acid sequences shown in Figures 9A-9C or contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In one embodiment of the present invention, the nucleic acid analog encodes a protein which has an amino acid sequence identical to that shown in Figures 10A-10C or encoded by the nucleic acid sequence contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In another embodiment, the nucleic acid analog encodes a protein having an amino acid sequence which differs from the amino acid sequences shown in Figures 10A-10C or encoded by the nucleic

-38-

acid contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In a further embodiment, the protein encoded by the nucleic acid analog has a function which is the same as the function of the receptor proteins having the amino acid sequence shown in Figures 10A-10C. In another embodiment, the function of the protein encoded by the nucleic acid analog differs from the function of the receptor protein having the amino acid sequence shown in Figures 10A-10C. In another embodiment, the variation in the nucleic acid sequence occurs within the transmembrane (TM) region of the protein. In a further embodiment, the variation in the nucleic acid sequence occurs outside of the TM region.

This invention provides the above-described isolated nucleic acid, wherein the nucleic acid is DNA. In an embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In still another embodiment, the nucleic acid is RNA. Methods for production and manipulation of nucleic acid molecules are well known in the art.

This invention further provides nucleic acid which is degenerate with respect to the DNA encoding any of the polypeptides described herein. In an embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence shown in Figures 1A-1C (SEQ ID NO: 1) or the nucleotide sequence contained in the plasmid pCDNA3.1-hSNORF36a-f (ATCC Accession No. 203977), that is, a nucleotide sequence which is translated into the same amino acid sequence. In another embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence shown in Figures 3A-3C (SEQ ID NO: 3) or the nucleotide sequence contained in the plasmid pCDNA3.1-hSNORF36b-f (ATCC Accession No. 203976), that is, a nucleotide sequence which is translated into the same amino acid sequence.

-39-

In another embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence shown in Figure 5 (SEQ ID NO: 5) or Figures 9A-9C (SEQ ID NO: 7) or the nucleotide sequence contained in the plasmids pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534) or pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216), that is, a nucleotide sequence which is translated into the same amino acid sequence.

This invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of the polypeptides of this invention, but which should not produce phenotypic changes.

Alternately, this invention also encompasses DNAs, cDNAs, and RNAs which hybridize to the DNA, cDNA, and RNA of the subject invention. Hybridization methods are well known to those of skill in the art.

The nucleic acids of the subject invention also include nucleic acid molecules coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs wherein one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors. The

-40-

creation of polypeptide analogs is well known to those of skill in the art (Spurney, R. F. et al. (1997); Fong, T.M. et al. (1995); Underwood, D.J. et al. (1994); Graziano, M.P. et al. (1996); Guan X.M. et al. (1995)).

5

The modified polypeptides of this invention may be transfected into cells either transiently or stably using methods well-known in the art, examples of which are disclosed herein. This invention also provides for binding assays using the modified polypeptides, in which the polypeptide is expressed either transiently or in stable cell lines. This invention further provides a compound identified using a modified polypeptide in a binding assay such as the binding assays described herein.

15

The nucleic acids described and claimed herein are useful for the information which they provide concerning the amino acid sequence of the polypeptide and as products for the large scale synthesis of the polypeptides by a variety of recombinant techniques. The nucleic acid molecule is useful for generating new cloning and expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

25

This invention also provides an isolated nucleic acid encoding species homologs of the SNORF36 receptor encoded by the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO: 1) or encoded by the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In one embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has substantially the same amino acid sequence as does the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In another embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog

30

35

-41-

which has above 75% amino acid identity to the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977); preferably above 85% amino acid identity to the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977); most preferably above 95% amino acid identity to the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In another embodiment, the mammalian SNORF36 receptor homolog has above 70% nucleic acid identity to the SNORF36 receptor gene contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977); preferably above 80% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977); more preferably above 90% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). Examples of methods for isolating and purifying species homologs are described elsewhere (e.g., U.S. Patent No. 5,602,024, WO94/14957, WO97/26853, WO98/15570).

This invention also provides an isolated nucleic acid encoding species homologs of the SNORF36 receptors encoded by the nucleic acid sequence shown in Figures 3A-3C (SEQ ID NO: 3) or encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In one embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has substantially the same amino acid sequence as does the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In another embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has above 75% amino acid identity to the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976); preferably above 85% amino acid identity to the SNORF36 receptor encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976); most preferably above 95% amino acid identity to the SNORF36

-42-

receptor encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In another embodiment, the mammalian SNORF36 receptor homolog has above 70% nucleic acid identity to the SNORF36 receptor gene contained in plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976); preferably above 80% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976); more preferably above 90% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976).

This invention also provides an isolated nucleic acid encoding species homologs of the SNORF36 receptors encoded by the nucleic acid sequence shown in Figure 5 (SEQ ID NO: 5) or encoded by the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). In one embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has substantially the same amino acid sequence as does the SNORF36 receptor encoded by the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). In another embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has above 75% amino acid identity to the SNORF36 receptor encoded by the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534); preferably above 85% amino acid identity to the SNORF36 receptor encoded by the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534); most preferably above 95% amino acid identity to the SNORF36 receptor encoded by the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). In another embodiment, the mammalian SNORF36 receptor homolog has above 70% nucleic acid identity to the SNORF36 receptor gene contained in plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534); preferably above 80% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534); more preferably above 90% nucleic acid identity to the SNORF36 receptor gene



-43-

contained in the plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534).

5 This invention also provides an isolated nucleic acid encoding species homologs of the SNORF36 receptors encoded by the nucleic acid sequence shown in Figures 9A-9C (SEQ ID NO: 7) or encoded by the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In one embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog  
10 which has substantially the same amino acid sequence as does the SNORF36 receptor encoded by the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In another embodiment, the nucleic acid encodes a mammalian SNORF36 receptor homolog which has above 75% amino acid identity to the SNORF36 receptor encoded by the pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216); preferably above 85% amino acid identity to the SNORF36 receptor encoded by the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216);  
15 most preferably above 95% amino acid identity to the SNORF36 receptor encoded by the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In another embodiment, the mammalian SNORF36 receptor homolog has above 70% nucleic acid identity to the SNORF36 receptor gene contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216);  
20 preferably above 80% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216); more preferably above 90% nucleic acid identity to the SNORF36 receptor gene contained in the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).  
25  
30

This invention provides an isolated nucleic acid encoding a modified mammalian SNORF36 receptor, which differs from a mammalian SNORF36 receptor by having an amino acid(s)  
35 deletion, replacement, or addition in the third intracellular domain.

-44-

This invention provides an isolated nucleic acid encoding a mammalian SNORF36 receptor. In one embodiment, the nucleic acid is DNA. In another embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In another  
5 embodiment, the nucleic acid is RNA.

In another embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor. In another embodiment, the human SNORF36a receptor has an amino acid sequence identical to  
10 that encoded by the pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In another embodiment, the human SNORF36a receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 2A-2B (SEQ ID NO: 2).

15 In another embodiment, the mammalian SNORF36 receptor is a human SNORF36b receptor. In another embodiment, the human SNORF36b receptor has an amino acid sequence identical to that encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In another embodiment, the human  
20 SNORF36b receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 4A-4C (SEQ ID NO: 4).

In an embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. In another embodiment, the rat SNORF36  
25 receptor has an amino acid sequence identical to that encoded by the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In another embodiment, the rat SNORF36 receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 10A-10C (SEQ ID NO: 8).

30 This invention provides a purified mammalian SNORF36 receptor protein. In one embodiment, the SNORF36 receptor protein is a human SNORF36a receptor protein. In another embodiment, the SNORF36 receptor protein is a human SNORF36b receptor  
35 protein. In a further embodiment, the SNORF36 receptor protein is a rat SNORF36 receptor protein.

-45-

This invention provides a vector comprising the nucleic acid of this invention. This invention further provides a vector adapted for expression in a cell which comprises the regulatory elements necessary for expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding the receptor so as to permit expression thereof, wherein the cell is a bacterial, amphibian, yeast, insect or mammalian cell. In one embodiment, the vector is a baculovirus. In another embodiment, the vector is a plasmid.

This invention provides a plasmid designated pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). This invention also provides a plasmid designated pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). This invention provides a plasmid designated pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534). This invention provides a plasmid designated pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

This invention further provides for any vector or plasmid which comprises modified untranslated sequences, which are beneficial for expression in desired host cells or for use in binding or functional assays. For example, a vector or plasmid with untranslated sequences of varying lengths may express differing amounts of the polypeptide depending upon the host cell used. In an embodiment, the vector or plasmid comprises the coding sequence of the polypeptide and the regulatory elements necessary for expression in the host cell.

This invention provides for a cell comprising the vector of this invention. In one embodiment, the cell is a non-mammalian cell. In one embodiment, the non-mammalian cell is a *Xenopus* oocyte cell or a *Xenopus* melanophore cell. In another embodiment, the cell is a mammalian cell. In another embodiment, the cell is a COS-7 cell, a 293 human embryonic

-46-

kidney cell, a NIH-3T3 cell, a LM(tk-) cell, a mouse Y1 cell, or a CHO cell. In another embodiment, the cell is an insect cell. In another embodiment, the insect cell is an Sf9 cell, an Sf21 cell or a Trichoplusia ni 5B-4 cell.

5

This invention provides a membrane preparation isolated from the cell in accordance with this invention.

10

Furthermore, this invention provides for a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within one of the two strands of the nucleic acid encoding the mammalian SNORF36 receptor contained in plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977), plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976), plasmid pEXJ.T3T7-rSNORF36p (ATCC Patent Depository No. PTA-534) or plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

20

25

This invention further provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO: 1) or (b) the reverse complement thereof. This invention further provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 3A-3C (SEQ ID NO: 3) or (b) the reverse complement thereof. This invention also provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein

30

35



5

15

20

25

30

35

subtypes within a family of receptors of which SNORF36 is a member.

25

25

-49-

Such transfected cells may also be used to test compounds and screen compound libraries to obtain compounds which bind to the SNORF36 receptor, as well as compounds which activate or inhibit activation of functional responses in such cells, and therefore are likely to do so in vivo. (See, for example, U.S. Patent Nos. 5,053,337; 5,155,218; 5,360,735; 5,472,866; 5,476,782; 5,516,653; 5,545,549; 5,556,753; 5,595,880; 5,602,024; 5,639,652; 5,652,113; 5,661,024; 5,766,879; 5,786,155; and 5,786,157, the disclosures of which are hereby incorporated by reference in their entirety into this application.)

This invention further provides an antibody capable of binding to a mammalian SNORF36 receptor encoded by a nucleic acid encoding a mammalian receptor. In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor.

This invention also provides an agent capable of competitively inhibiting the binding of the antibody to a mammalian SNORF36 receptor. In one embodiment, the antibody is a monoclonal antibody or antisera.

Methods of preparing and employing antisense oligonucleotides, antibodies, nucleic acid probes and transgenic animals directed to the SNORF36 receptor are well known in the art. (See, for example, U.S. Patent Nos. 5,053,337; 5,155,218; 5,360,735; 5,472,866; 5,476,782; 5,516,653; 5,545,549; 5,556,753; 5,595,880; 5,602,024; 5,639,652; 5,652,113; 5,661,024; 5,766,879; 5,786,155; and 5,786,157, the disclosures of which are hereby incorporated by reference in their entirety into this application.)

This invention provides for an antisense oligonucleotide having a sequence capable of specifically hybridizing to RNA

-50-

encoding a mammalian SNORF36 receptor, so as to prevent translation of such RNA. This invention further provides for an antisense oligonucleotide having a sequence capable of specifically hybridizing to genomic DNA encoding a mammalian SNORF36 receptor, so as to prevent transcription of such genomic DNA. In one embodiment, the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

10 This invention also provides for an antibody capable of binding to a mammalian SNORF36 receptor encoded by a nucleic acid in accordance with this invention. In an embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the  
15 mammalian SNORF36 receptor is a rat SNORF36 receptor.

Moreover, this invention provides an agent capable of competitively inhibiting the binding of an antibody in accordance with this invention to a mammalian SNORF36  
20 receptor. In one embodiment, the antibody is a monoclonal antibody or antisera.

This invention still further provides a pharmaceutical composition comprising (a) an amount of an oligonucleotide in accordance with this invention capable of passing through  
25 a cell membrane and effective to reduce expression of a mammalian SNORF36 receptor and (b) a pharmaceutically acceptable carrier capable of passing through the cell membrane.

30 In one embodiment, the oligonucleotide is coupled to a substance which inactivates mRNA. In another embodiment, the substance which inactivates mRNA is a ribozyme. In another embodiment, the pharmaceutically acceptable carrier comprises  
35 a structure which binds to a mammalian SNORF36 receptor on a cell capable of being taken up by the cells after binding



-51-

to the structure. In another embodiment, the pharmaceutically acceptable carrier is capable of binding to a mammalian SNORF36 receptor which is specific for a selected cell type.

5

This invention also provides a pharmaceutical composition which comprises an amount of an antibody in accordance with this invention effective to block binding of a ligand to a human SNORF36a receptor or a human SNORF36b receptor and a pharmaceutically acceptable carrier.

10

This invention further provides a transgenic, nonhuman mammal expressing DNA encoding a mammalian SNORF36 receptor in accordance with this invention. This invention provides a transgenic, nonhuman mammal comprising a homologous recombination knockout of a native mammalian SNORF36 receptor. This invention further provides a transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a mammalian SNORF36 receptor in accordance with this invention so placed within such genome as to be transcribed into antisense mRNA which is complementary and hybridizes with mRNA encoding the mammalian SNORF36 receptor so as to thereby reduce translation or such mRNA and expression of such receptor. In one embodiment, the DNA encoding the mammalian SNORF36 receptor additionally comprises an inducible promoter. In another embodiment, the DNA encoding the mammalian SNORF36 receptor additionally comprises tissue specific regulatory elements. In another embodiment, the transgenic, nonhuman mammal is a mouse.

20  
25  
30

This invention provides for a process for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises contacting cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the

35

-52-

compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor. This invention further provides for a process for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises contacting a membrane preparation from cells containing DNA encoding and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor.

In an embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In another embodiment, the mammalian SNORF36 receptor has substantially the same amino acid sequence as the human SNORF36a receptor encoded by plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. 203977). In another embodiment, the mammalian SNORF36 receptor has substantially the same amino acid sequence as the human SNORF36b receptor encoded by plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. 203976). In another embodiment, the mammalian SNORF36 receptor has substantially the same amino acid sequence as that shown in Figures 2A-2B (SEQ ID NO: 2) or Figures 4A-4C (SEQ ID NO: 4). In another embodiment, the mammalian SNORF36 receptor has the amino acid sequence shown in Figures 2A-2B (SEQ ID NO: 2) or Figures 4A-4C (SEQ ID NO: 4).

In another embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. In another embodiment, the mammalian SNORF36 receptor has substantially the same amino acid sequence as the rat SNORF36 receptor encoded by plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216). In another embodiment, the mammalian SNORF36 receptor has substantially the same amino acid sequence as that shown in

-53-

Figures 10A-10C (SEQ ID NO: 8). In another embodiment, the mammalian SNORF36 receptor has the amino acid sequence shown in Figures 10A-10C (SEQ ID NO: 8).

5 In one embodiment, the compound is not previously known to bind to a mammalian SNORF36 receptor. In one embodiment, the cell is an insect cell. In one embodiment, the cell is a mammalian cell. In another embodiment, the cell is nonneuronal in origin. In another embodiment, the  
10 nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell. In another embodiment, the compound is a compound not previously known to bind to a mammalian SNORF36 receptor. This invention provides a compound identified by  
15 the preceding process according to this invention.

This invention still further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which  
20 comprises separately contacting cells expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the  
25 second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF36 receptor in the  
30 presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF36 receptor.

This invention provides a process involving competitive  
35 binding for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which

comprises separately contacting a membrane preparation from cells expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF36 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF36 receptor.

In an embodiment of the present invention, the second chemical compound is a retinoic acid derivative. Examples of retinoic acid derivatives include, but are not limited to, all-trans retinoic acid (ATRA), 9-cis-retinal, 13-cis-retinal, and all-trans-retinal.

In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In another embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. In a further embodiment, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In another embodiment, the cell is nonneuronal in origin. In another embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell. In another embodiment, the compound is not previously known to bind to a mammalian SNORF36 receptor. This invention provides for a compound identified by the preceding process according to this invention.

This invention provides for a method of screening a plurality

-55-

of chemical compounds not known to bind to a mammalian SNORF36 receptor to identify a compound which specifically binds to the mammalian SNORF36 receptor, which comprises (a) contacting cells transfected with, and expressing, DNA encoding the mammalian SNORF36 receptor with a compound known to bind specifically to the mammalian SNORF36 receptor; (b) contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian SNORF36 receptor, under conditions permitting binding of compounds known to bind to the mammalian SNORF36 receptor; (c) determining whether the binding of the compound known to bind to the mammalian SNORF36 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the mammalian SNORF36 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF36 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF36 receptor to identify a compound which specifically binds to the mammalian SNORF36 receptor, which comprises (a) contacting a membrane preparation from cells transfected with, and expressing, DNA encoding the mammalian SNORF36 receptor with the plurality of compounds not known to bind specifically to the mammalian SNORF36 receptor under conditions permitting binding of compounds known to bind to the mammalian SNORF36 receptor; (b) determining whether the binding of a compound known to bind to the mammalian SNORF36 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (c) separately determining the binding to the mammalian SNORF36 receptor of each compound included in the plurality of

-56-

compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF36 receptor.

5 In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. In another embodiment, the cell is a mammalian cell. In another embodiment, the mammalian cell is non-  
10 neuronal in origin. In a further embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell, or an NIH-3T3 cell.

15 This invention also provides a method of detecting expression of a mammalian SNORF36 receptor by detecting the presence of mRNA coding for the mammalian SNORF36 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with a nucleic acid probe according to  
20 this invention under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the mammalian SNORF36 receptor by the cell.

25 This invention further provides for a method of detecting the presence of a mammalian SNORF36 receptor on the surface of a cell which comprises contacting the cell with an antibody according to this invention under conditions permitting binding of the antibody to the receptor, detecting the  
30 presence of the antibody bound to the cell, and thereby detecting the presence of the mammalian SNORF36 receptor on the surface of the cell.

35 This invention still further provides a method of determining the physiological effects of varying levels of activity of mammalian SNORF36 receptors which comprises producing a

-57-

transgenic, nonhuman mammal in accordance with this invention whose levels of mammalian SNORF36 receptor activity are varied by use of an inducible promoter which regulates mammalian SNORF36 receptor expression.

5

This invention additionally provides a method of determining the physiological effects of varying levels of activity of mammalian SNORF36 receptors which comprises producing a panel of transgenic, nonhuman mammals in accordance with this invention each expressing a different amount of mammalian SNORF36 receptor.

10

Moreover, this invention provides method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor comprising administering a compound to a transgenic, nonhuman mammal according to this invention, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal as a result of overactivity of a mammalian SNORF36 receptor, the alleviation of such an abnormality identifying the compound as an antagonist. In an embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. The invention also provides an antagonist identified by the preceding method according to this invention. This invention further provides a composition, e.g. a pharmaceutical composition comprising an antagonist according to this invention and a carrier, e.g. a pharmaceutically acceptable carrier. This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition according to this invention so as to thereby

15

20

25

30

35

-58-

treat the abnormality.

In addition, this invention provides a method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor comprising administering a compound to a transgenic, nonhuman mammal according to this invention, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal, the alleviation of such an abnormality identifying the compound as an agonist. In an embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. This invention provides an agonist identified by the preceding method according to this invention. This invention provides a composition, e.g. a pharmaceutical composition comprising an agonist identified by a method according to this invention and a carrier, e.g. a pharmaceutically acceptable carrier.

Moreover, this invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition of this invention so as to thereby treat the abnormality.

Yet further, this invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific mammalian allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b) performing a restriction digest of the DNA with a panel of restriction enzymes; (c) electrophoretically separating the resulting DNA fragments on a sizing gel; (d) contacting the resulting gel with a nucleic acid probe capable of



specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian SNORF36 receptor and labeled with a detectable marker; (e) detecting labeled bands which have hybridized to the DNA encoding a mammalian SNORF36 receptor to create a unique band pattern specific to the DNA of subjects suffering from the disorder; (f) repeating steps (a)-(e) with DNA obtained for diagnosis from subjects not yet suffering from the disorder; and (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) with the band pattern from step (f) for subjects not yet suffering from the disorder so as to determine whether the patterns are the same or different and thereby diagnose predisposition to the disorder if the patterns are the same.

In one embodiment, the disorder is a disorder associated with the activity of a specific mammalian allele is diagnosed.

This invention also provides a method of preparing a purified mammalian SNORF36 receptor according to this invention which comprises: (a) culturing cells which express the mammalian SNORF36 receptor; (b) recovering the mammalian SNORF36 receptor from the cells; and (c) purifying the mammalian SNORF36 receptor so recovered.

This invention further provides a method of preparing a purified mammalian SNORF36 receptor according to this invention which comprises: (a) inserting a nucleic acid encoding the mammalian SNORF36 receptor into a suitable expression vector; (b) introducing the resulting vector into a suitable host cell; (c) placing the resulting host cell in suitable condition permitting the production of the mammalian SNORF36 receptor; (d) recovering the mammalian SNORF36 receptor so produced; and optionally (e) isolating and/or purifying the mammalian SNORF36 receptor so recovered.

-60-

Furthermore, this invention provides a process for determining whether a chemical compound is a mammalian SNORF36 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF36 receptor with the compound under conditions permitting the activation of the mammalian SNORF36 receptor, and detecting any increase in mammalian SNORF36 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF36 receptor agonist.

This invention also provides a process for determining whether a chemical compound is a mammalian SNORF36 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF36 receptor with the compound in the presence of a known mammalian SNORF36 receptor agonist, under conditions permitting the activation of the mammalian SNORF36 receptor, and detecting any decrease in mammalian SNORF36 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF36 receptor antagonist.

In an embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In another embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor.

This invention still further provides a composition, for example a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor agonist determined by a process according to this invention effective to increase activity of a mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF36 receptor agonist is not previously known.

Also, this invention provides a composition, for example a

-61-

pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor antagonist determined by a process according to this invention effective to reduce activity of a mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF36 receptor antagonist is not previously known.

This invention moreover provides a process for determining whether a chemical compound specifically binds to and activates a mammalian SNORF36 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the chemical compound under conditions suitable for activation of the mammalian SNORF36 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change, e.g. an increase, in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian SNORF36 receptor.

In one embodiment, the second messenger response comprises chloride channel activation and the change in second messenger is an increase in the level of chloride current. In another embodiment, the second messenger response comprises change in intracellular calcium levels and the change in second messenger is an increase in the measure of intracellular calcium. In another embodiment, the second messenger response comprises release of inositol phosphate and the change in second messenger is an increase in the level of inositol phosphate. In another embodiment, the second messenger response comprises release of arachidonic acid and the change in second messenger is an increase in the level of arachidonic acid. In yet another embodiment, the second messenger response comprises GTPγS ligand binding and

-62-

the change in second messenger is an increase in GTPyS ligand binding. In another embodiment, the second messenger response comprises activation of MAP kinase and the change in second messenger response is an increase in MAP kinase activation. In a further embodiment, the second messenger response comprises cAMP accumulation and the change in second messenger response is a reduction in cAMP accumulation.

This invention still further provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian SNORF36 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to activate the mammalian SNORF36 receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian SNORF36 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change, e.g. increase, in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian SNORF36 receptor.

In an embodiment of the present invention, the second chemical compound is a retinoic acid derivative. Examples of retinoic acid derivatives include, but are not limited to, all-trans retinoic acid (ATRA), 9-cis-retinal, 13-cis-retinal, and all-trans-retinal.

-63-

In one embodiment, the second messenger response comprises chloride channel activation and the change in second messenger response is a smaller increase in the level of chloride current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger response comprises change in intracellular calcium levels and the change in second messenger response is a smaller increase in the measure of intracellular calcium in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger response comprises release of inositol phosphate and the change in second messenger response is a smaller increase in the level of inositol phosphate in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

In one embodiment, the second messenger response comprises activation of MAP kinase and the change in second messenger response is a smaller increase in the level of MAP kinase activation in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger response comprises change in cAMP levels and the change in second messenger response is a smaller change in the level of cAMP in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger response comprises release of arachidonic acid and the change in second messenger response is an increase in the level of arachidonic acid levels in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In a further embodiment, the

-64-

second messenger response comprises GTPyS ligand binding and the change in second messenger is a smaller increase in GTPyS ligand binding in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor. In another embodiment, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In another embodiment, the mammalian cell is nonneuronal in origin. In another embodiment, the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell. In another embodiment, the compound is not previously known to bind to a mammalian SNORF36 receptor.

Further, this invention provides a compound determined by a process according to this invention and a composition, for example, a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor agonist determined to be such by a process according to this invention effective to increase activity of the mammalian SNORF36 receptor and a carrier, for example, a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF36 receptor agonist is not previously known.

This invention also provides a composition, for example, a pharmaceutical composition, which comprises an amount of a mammalian SNORF36 receptor antagonist determined to be such by a process according to this invention, effective to reduce activity of the mammalian SNORF36 receptor and a carrier, for example a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF36 receptor antagonist is not

previously known.

This invention yet further provides a method of screening a plurality of chemical compounds not known to activate a mammalian SNORF36 receptor to identify a compound which activates the mammalian SNORF36 receptor which comprises:

5 (a) contacting cells transfected with and expressing the mammalian SNORF36 receptor with the plurality of compounds not known to activate the mammalian SNORF36 receptor, under

10 conditions permitting activation of the mammalian SNORF36 receptor; (b) determining whether the activity of the mammalian SNORF36 receptor is increased in the presence of one or more of the compounds; and if so (c) separately

15 determining whether the activation of the mammalian SNORF36 receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian SNORF36 receptor. In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further

20 embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian SNORF36 receptor to identify a compound which inhibits the activation of the mammalian SNORF36 receptor, which comprises: (a) contacting cells transfected with and expressing the mammalian SNORF36 receptor with the plurality of compounds in the presence of a known mammalian SNORF36

25 receptor agonist, under conditions permitting activation of the mammalian SNORF36 receptor; (b) determining whether the extent or amount of activation of the mammalian SNORF36 receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of

30 the mammalian SNORF36 receptor in the absence of such one or more compounds; and if so (c) separately determining whether

35

-66-

each such compound inhibits activation of the mammalian SNORF36 receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of compounds which inhibits the activation  
5 of the mammalian SNORF36 receptor.

In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In a further embodiment, the mammalian SNORF36 receptor is a rat SNORF36  
10 receptor. In another embodiment, wherein the cell is a mammalian cell. In another embodiment, the mammalian cell is non-neuronal in origin. In another embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

15 This invention also provides a composition, for example, a pharmaceutical composition, comprising a compound identified by a method according to this invention in an amount effective to increase mammalian SNORF36 receptor activity and  
20 a carrier, for example, a pharmaceutically acceptable carrier.

This invention still further provides a composition, for example, a pharmaceutical composition, comprising a compound  
25 identified by a method according to this invention in an amount effective to decrease mammalian SNORF36 receptor activity and a carrier, for example, a pharmaceutically acceptable carrier.

30 Furthermore, this invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject a compound which is a mammalian SNORF36 receptor agonist in an  
35 amount effective to treat the abnormality. In one embodiment, the abnormality is a regulation of a steroid



-67-

hormone disorder, an epinephrine release disorder, a gastrointestinal disorder, a cardiovascular disorder, an electrolyte balance disorder, hypertension, diabetes, a respiratory disorder, asthma, a reproductive function disorder, an immune disorder, an endocrine disorder, a musculoskeletal disorder, a neuroendocrine disorder, a cognitive disorder, a memory disorder, somatosensory and neurotransmission disorders, metabolic disorders, a motor coordination disorder, a sensory integration disorder, a motor integration disorder, a dopaminergic function disorder, an appetite disorder, such as anorexia or obesity, a sensory transmission disorder, drug addiction, an olfaction disorder, an autonomic nervous system disorder, pain, neuropsychiatric disorders, affective disorder, migraine, circadian disorders, visual disorders, urinary disorders, blood coagulation-related disorders, developmental disorders, or ischemia-reperfusion injury-related diseases.

In a further embodiment of the present invention, the abnormality is a gestational abnormality, a sleep disorder such as insomnia, jet lag or shift-related conditions, disorders associated with melatonin release, and disorders associated with choroid plexus function.

This invention additionally provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject a compound which is a mammalian SNORF36 receptor antagonist in an amount effective to treat the abnormality. In one embodiment, the abnormality is a regulation of a steroid hormone disorder, an epinephrine release disorder, a gastrointestinal disorder, a cardiovascular disorder, an electrolyte balance disorder, hypertension, diabetes, a respiratory disorder, asthma, a reproductive function disorder, an immune disorder, an endocrine disorder, a

-68-

musculoskeletal disorder, a neuroendocrine disorder, a cognitive disorder, a memory disorder, somatosensory and neurotransmission disorders, metabolic disorders, a motor coordination disorder, a sensory integration disorder, a motor integration disorder, a dopaminergic function disorder, an appetite disorder, such as anorexia or obesity, a sensory transmission disorder, drug addiction, an olfaction disorder, an autonomic nervous system disorder, pain, neuropsychiatric disorders, affective disorder, migraine, circadian disorders, visual disorders, urinary disorders, blood coagulation-related disorders, developmental disorders, or ischemia-reperfusion injury-related diseases.

In a further embodiment of the present invention, the abnormality is a gestational abnormality, a sleep disorder such as insomnia, jet lag or shift-related conditions, disorders associated with melatonin release, and disorders associated with choroid plexus function.

This invention also provides a process for making a composition of matter which specifically binds to a mammalian SNORF36 receptor which comprises identifying a chemical compound using a process in accordance with this invention and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof. In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In another embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor.

This invention further provides a process for preparing a composition, for example a pharmaceutical composition which comprises admixing a carrier, for example, a pharmaceutically acceptable carrier, and a pharmaceutically effective amount of a chemical compound identified by a process in accordance with this invention or a novel structural and functional

-69-

analog or homolog thereof. In one embodiment, the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor. In another embodiment, the mammalian SNORF36 receptor is a rat SNORF36 receptor.

5

Thus, once the gene for a targeted receptor subtype is cloned, it is placed into a recipient cell which then expresses the targeted receptor subtype on its surface. This cell, which expresses a single population of the targeted human receptor subtype, is then propagated resulting in the establishment of a cell line. This cell line, which constitutes a drug discovery system, is used in two different types of assays: binding assays and functional assays. In binding assays, the affinity of a compound for both the receptor subtype that is the target of a particular drug discovery program and other receptor subtypes that could be associated with side effects are measured. These measurements enable one to predict the potency of a compound, as well as the degree of selectivity that the compound has for the targeted receptor subtype over other receptor subtypes. The data obtained from binding assays also enable chemists to design compounds toward or away from one or more of the relevant subtypes, as appropriate, for optimal therapeutic efficacy. In functional assays, the nature of the response of the receptor subtype to the compound is determined. Data from the functional assays show whether the compound is acting to inhibit or enhance the activity of the receptor subtype, thus enabling pharmacologists to evaluate compounds rapidly at their ultimate human receptor subtypes targets permitting chemists to rationally design drugs that will be more effective and have fewer or substantially less severe side effects than existing drugs.

15

20

25

30

35

Approaches to designing and synthesizing receptor subtype-selective compounds are well known and include traditional medicinal chemistry and the newer technology of combinatorial

-70-

chemistry, both of which are supported by computer-assisted molecular modeling. With such approaches, chemists and pharmacologists use their knowledge of the structures of the targeted receptor subtype and compounds determined to bind and/or activate or inhibit activation of the receptor subtype to design and synthesize structures that will have activity at these receptor subtypes.

Combinatorial chemistry involves automated synthesis of a variety of novel compounds by assembling them using different combinations of chemical building blocks. The use of combinatorial chemistry greatly accelerates the process of generating compounds. The resulting arrays of compounds are called libraries and are used to screen for compounds ("lead compounds") that demonstrate a sufficient level of activity at receptors of interest. Using combinatorial chemistry it is possible to synthesize "focused" libraries of compounds anticipated to be highly biased toward the receptor target of interest.

Once lead compounds are identified, whether through the use of combinatorial chemistry or traditional medicinal chemistry or otherwise, a variety of homologs and analogs are prepared to facilitate an understanding of the relationship between chemical structure and biological or functional activity. These studies define structure activity relationships which are then used to design drugs with improved potency, selectivity and pharmacokinetic properties. Combinatorial chemistry is also used to rapidly generate a variety of structures for lead optimization. Traditional medicinal chemistry, which involves the synthesis of compounds one at a time, is also used for further refinement and to generate compounds not accessible by automated techniques. Once such drugs are defined the production is scaled up using standard chemical manufacturing methodologies utilized throughout the pharmaceutical and chemistry industry.

-71-

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

5

## EXPERIMENTAL DETAILS

### Materials and Methods

#### 5 Identification of a fragment of the human SNORF36 receptor

A human placental genomic phage library (2.75 million recombinants, Stratagene, LaJolla, CA) was screened using <sup>32</sup>P-labeled oligonucleotide probes, RW76, RW77, RW96, RW97, RW98 and RW99, designed against transmembrane (TM) domains  
10 III, V and VI of the human serotonin 5-HT<sub>1</sub> receptor. The overlapping oligonucleotide probes were labeled with DNA Polymerase I (Klenow Fragment) and [α-<sup>32</sup>P]dATP/dCTP.

Hybridization of nitrocellulose filter overlays of the plates  
15 was performed at low stringency: 40°C in a solution containing 25% formamide, 5x SSC (1x SSC is 0.15M sodium chloride, 0.015M sodium citrate), 1x Denhardt's solution (0.02% polyvinylpyrrolindone, 0.02% Ficoll, 0.02% bovine serum albumin), 7 mM Tris and 25 µg/ml sonicated salmon sperm  
20 DNA. The filters were washed at 40 °C in 0.1x SSC containing 0.1% sodium dodecyl sulfate and exposed at -70°C to Kodak XAR film in the presence of intensifying screens.

A positive signal on plate 10 was isolated on a secondary  
25 plating. A 2.3 kb fragment, from a *Hind*III/*Xba*I digest of DNA isolated from this positive, was identified by Southern blot analysis, subcloned into pUC18 (Gibco BRL, Gaithersburg, MD) and used to transform E.Coli XL1 Blue cells (Stratagene, La Jolla, CA). Plasmid DNA from one transformant, K39, was  
30 sequenced using the Sanger dideoxy nucleotide chain termination method (Sanger et al., 1977) on denatured double-stranded plasmid templates, using Sequenase (US Biochemical Corp., Cleveland, OH). Analysis of the sequence of K39 revealed TMs III and IV of a novel GPCR with highest homology  
35 to adrenergic receptors.

-73-

Isolation of a full-length human SNORF36 receptor

To isolate a full-length SNORF36 receptor, pools of a human hippocampal cDNA library were screened by polymerase chain reaction (PCR) using T604, a K39 specific primer, and either T94, a vector primer, or T603, a K39 specific primer. PCR was performed with Taq DNA Polymerase (Roche Molecular Biochemicals, Indianapolis, IN) with the following protocol: 94°C hold for 5 minutes; 40 cycles of 94°C for 2 minute, 72°C for 4 minutes; 10 minute hold at 72°C; 4°C hold until the samples are run on a gel. High stringency hybridization of isolated colonies from two positive pools with T605, a K39-specific oligonucleotide probe, and subsequent PCR testing of positive colonies, resulted in the isolation of a single positive clone named TL252. Analysis of the sequence of TL252 revealed that it contained TMI-TMVII, but was missing the NH<sub>2</sub> and COOH termini.

To isolate the COOH termini of SNORF36, 3' Rapid Amplification of cDNA Ends (RACE), was performed using the Clontech Marathon cDNA Amplification kit (Clontech, Palo Alto, CA). Using the supplier's protocol, Marathon adapters were ligated onto ds cDNA prepared from human hippocampal polyA<sup>+</sup> RNA. The initial PCR was performed with the supplier's Adapter Primer 1 and A48, a forward primer from TMVI of TL252. 2 µls of this initial PCR reaction was re-amplified using the Adaptor Primer 2 and A49, a forward primer from TMVI. PCR was performed with Advantage KlenTaq Polymerase (Clontech, Palo Alto, CA) under the following conditions: 30 seconds at 94° C; 30 cycles of 94° C for 30 seconds, 68° C 4 minutes; and 4° C hold until the products were ready for analysis. A 1.5 kb fragment was isolated from an agarose TAE gel using the QIAQUICK gel extraction kit (QIAGEN, Chatsworth, CA) and subcloned into the TA cloning vector (Invitrogen, San Diego, CA). One transformant, AB25, was sequenced using the ABI Big Dye cycle sequencing protocol and

-74-

ABI 377 sequencers (ABI, Foster City, CA). Sequences were analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, WI.). Analysis of the sequence of AB25 revealed the COOH terminus and a stop codon approximately 350  
5 bp downstream from TMVII.

To look for a full-length SNORF36 cDNA, pools of a human hippocampal cDNA library were screened by PCR with TL252-specific primers BB788 and BB789 using the Expand Long  
10 Template PCR System (Boehringer-Mannheim, Indianapolis, IN). Conditions for PCR amplification were as follows: 94°C hold for 5 minutes; 40 cycles of 94°C for 30 seconds, 68°C for 2 minutes; 68°C hold for 5 minutes; 4°C hold until ready for agarose gel electrophoresis. This screen yielded three  
15 positive pools. Subsequent high-stringency hybridization of isolated colonies from two of these pools using a [ $\gamma$ -<sup>32</sup>P]-ATP-labeled SNORF36-specific probe (BB791) resulted in the identification of 2 positive individual colonies, 260.13.1 and 243.33.3. Sequencing of these clones revealed that they  
20 were identical and were full-length at the 3' end. However, while these clones contained some sequence upstream of TMI, they did not contain the initiating methionine. In addition, these clones both contained an 11 amino acid insert in the first intracellular loop that was not present in TL252.

25 To identify the NH<sub>2</sub> terminal sequence, 5'RACE was performed on Marathon-Ready human hippocampal cDNA (Clontech, Palo Alto, CA) according to the Marathon cDNA Amplification Kit protocol. The initial PCR was performed with the supplier's  
30 Adapter Primer 1 and BB798 a reverse primer from the second intracellular loop of 260.13.1. One  $\mu$ l of this initial PCR reaction was re-amplified using the Adaptor Primer 2 and BB797, a reverse primer from TMI. PCR was performed with Advantage KlenTaq Polymerase (Clontech, Palo Alto, CA) under  
35 the following conditions: 30 seconds at 94°C; 5 cycles of 94°C for 30 seconds and 72°C for 3 minutes; 5 cycles of 94°C



-75-

for 30 seconds and 70°C for 3 minutes; 23 cycles (initial PCR) or 20 cycles (nested PCR) of 94°C for 30 seconds and 68°C for 3 minutes; 68°C hold for 7 minutes, and 4°C hold until the products were ready for analysis. A 500 bp fragment was isolated from a 1% agarose TAE gel using the QIAQUICK gel extraction kit (QIAGEN, Chatsworth, CA) and sequenced using ABI 377 sequencers and BigDye termination cycle sequencing as described above. Analysis of this sequence revealed the NH<sub>2</sub> terminus including 2 methionines 71 and 52 amino acids upstream from TMI.

A full-length cDNA clone for SNORF36a (without the 11 amino acid insert) was constructed as follows. The 5' end of the cDNA was amplified from hippocampal and pituitary cDNAs using the Expand Long Template PCR System (Roche Molecular Biochemicals, Indianapolis, IN) and BB812, a forward primer from the 5'UT also incorporating a *Bam*HI restriction site, and BB813, a reverse primer from the third extracellular loop. Conditions for PCR amplification were as follows: 94°C hold for 5 minutes; 37 cycles of 94°C for 30 seconds; 68°C for 2.5 minutes; 68°C hold for 7 minutes; 4°C hold until ready for agarose gel electrophoresis. 1050 bp bands from 9 independent PCR reactions were cut from a 1% agarose gel, purified using the QIAQUICK gel extraction kit (QIAGEN, Chatsworth, CA), subcloned into the TA cloning vector (Invitrogen, San Diego, CA) and sequenced using the ABI Big Dye cycle sequencing protocol and ABI 377 sequencers (ABI, Foster City, CA). Analysis of these sequences revealed the presence of an allelic variation. Nucleotide 39 (Figures 1A-C and 3A-C) is either an adenine or a guanine. One of these PCR products, F9, matched the consensus sequence with the exception of a single conservative nucleotide change. A 1021 bp *Bam*HI/*Bgl*II fragment from F9 was then ligated along with a 1180 bp *Bgl*II/*Eco*RI fragment from the human hippocampal library pool 260.13.1 into a *Bam*HI/*Eco*RI-cut pcDNA3.1

-76-

(Invitrogen, San Diego, CA), and this construct was named BO108. The single point mutation introduced by the PCR fragment was corrected with the QuikChange Site-Directed Mutagenesis Kit (Stratagene, LaJolla, CA) using BB926 and BB927 and the manufacturer's instructions. The resulting SNORF36a construct, BO109, was sequenced on both strands as described above. This clone was renamed pcDNA3.1-hSNORF36a-f. A full-length clone for SNORF36b (with the 11 amino acid insert) was constructed as follows. A 1260 bp fragment was obtained by amplifying BO108 with BB796, a forward primer in TMI and BB936, a reverse primer at the stop codon also incorporating a *HindIII* site, with the following protocol: 94°C hold for 5 minutes; 32 cycles of 94°C for 30 seconds; 68°C for 2.5 minutes; 68°C hold for 7 minutes; 4°C hold until ready for agarose gel electrophoresis. A 1130 bp *NspI/HindIII* fragment from this PCR product and a 390 bp *BamHI/NspI* fragment from K81, one of the PCR products from the BB812-BB813 PCR described above, was ligated into a *BamHI/HindIII*-cut pcDNA3.1 (Invitrogen, San Diego, CA). The resulting SNORF36b construct, BO110, was sequenced on both strands as described above. This clone was renamed pcDNA3.1-hSNORF36b-f.

#### Isolation of a Fragment of the Rat Homologue of SNORF36

To obtain a fragment of the rat homologue of SNORF36, 100 ng of rat genomic DNA (Clontech, Palo Alto, CA) was amplified with BB788, a forward PCR primer corresponding to TMIII of human SNORF36 and BB1097, a reverse primer corresponding to TMV of human SNORF36. PCR was performed with the Expand Long Template PCR System (Roche Molecular Biochemicals) under the following conditions: 30 seconds at 94°C, 45 seconds at 45°C or 50°C, 1.5 minutes at 68°C for 40 cycles, with a pre- and post-incubation of 5 minutes at 94°C and 7 minutes at 68°C respectively. Bands of 800 bp from 7 independent PCR reactions were isolated from a TAE gel, purified using the

-77-

QIAQUICK gel extraction kit (QIAGEN, Chatsworth, CA), and sequenced on both strands as described above. Analysis of the sequence revealed an intron in the second extracellular loop. The consensus of the 7 sequences was used to design forward and reverse PCR primers (BB1182, also incorporating a *Bam*HI restriction site, and BB1183, also incorporating a *Hind*III site) which were used to amplify a band from rat spinal cord cDNA using the following conditions: 30 seconds at 94°C, 30 seconds at 64°C, 1.5 minutes at 68°C for 37 cycles, with a pre- and post-incubation of 5 minutes at 94°C and 7 minutes at 68°C respectively. Products from 3 independent PCR reactions were digested with *Bam*HI and *Hind*III and fragments of 250 bp were gel-purified and ligated into the expression vector pEXJ.T3T7. One transformant from each PCR reaction was sequenced as above. The nucleotide sequences of two products were identical to the consensus and one, KO56, was renamed pEXJ-rSNORF36-p.

#### Isolation of a full-length rat SNORF36 receptor

To look for the full-length rat SNORF36 cDNA, pools of a rat spinal cord cDNA library were screened by PCR with BB1182, a forward primer from TMIII of the rat SNORF36 fragment, and BB1183, a reverse primer from TMV of the rat SNORF36 fragment. PCR was performed using the Expand Long Template PCR System (Roche Molecular Biochemicals, Indianapolis, IN) under the following conditions: 94°C hold for 5 minutes; 40 cycles of 94°C for 30 seconds, 68°C for 5 minutes; 68°C hold for 7 minutes; 4°C hold until ready for agarose gel electrophoresis. This screen yielded 2 positive pools. Subsequent high-stringency hybridization of isolated colonies from 1 of these pools using a [ $\gamma$ -<sup>32</sup>P]-ATP-labeled rat SNORF36-specific probe (BB1298) resulted in the identification of a positive individual colony, N323.16.1F, renamed KO109. Sequencing of KO109 revealed that it contained an insert of 2.6 kb, including an open reading

-78-

frame of 1422 nucleotides, 148 nucleotides of 5'UT and approximately 1000 nucleotides of 3'UT. This clone was renamed pEXJ.T7-rSNORF36-f.

5     Isolation of other species homologs of SNORF36 receptor cDNA

A nucleic acid sequence encoding a SNORF36 receptor cDNA from other species may be isolated using standard molecular biology techniques and approaches such as those described below:

10

Approach #1: A genomic library (e.g., cosmid, phage, P1, BAC, YAC) generated from the species of interest may be screened with a <sup>32</sup>P-labeled oligonucleotide probe corresponding to a fragment of the human or rat SNORF36 receptors whose sequence is shown in Figures 1A-1C, 3A-3C, 5 or 9A-9C to isolate a genomic clone. The full-length sequence may be obtained by sequencing this genomic clone. If one or more introns are present in the gene, the full-length intronless gene may be obtained from cDNA using standard molecular biology techniques. For example, a forward PCR primer designed in the 5'UT and a reverse PCR primer designed in the 3'UT may be used to amplify a full-length, intronless receptor from cDNA. Standard molecular biology techniques could be used to subclone this gene into a mammalian expression vector.

25

Approach #2: Standard molecular biology techniques may be used to screen commercial cDNA phage libraries of the species of interest by hybridization under reduced stringency with a <sup>32</sup>P-labeled oligonucleotide probe corresponding to a fragment of the sequences shown in Figures 1A-1C, 3A-3C, 5 or 9A-9C. One may isolate a full-length SNORF36 receptor by obtaining a plaque purified clone from the lambda libraries and then subjecting the clone to direct DNA sequencing. Alternatively, standard molecular biology techniques could be used to screen cDNA plasmid libraries by PCR amplification

35

-79-

of library pools using primers designed against a partial species homolog sequence. A full-length clone may be isolated by Southern hybridization of colony lifts of positive pools with a <sup>32</sup>P-oligonucleotide probe.

5

Approach #3: 3' and 5' RACE may be utilized to generate PCR products from cDNA derived from the species of interest expressing SNORF36 which contain the additional sequence of SNORF36. These RACE PCR products may then be sequenced to  
10 determine the additional sequence. This new sequence is then used to design a forward PCR primer in the 5'UT and a reverse primer in the 3'UT. These primers are then used to amplify a full-length SNORF36 clone from cDNA.

15

Examples of other species include, but are not limited to, mouse, dog, monkey, hamster and guinea pig.

Primers and probes used in the identification of SNORF36:

RW76: 5' - CATCGCCCTCGACGTGCTGTGCTGCACCTCATCCATCT

20

TGCACCT -3' (SEQ ID NO: 9)

RW77: 5' - CATGGACAGGTGCGCTACCGCGTGTCCACGTTCTACC

TACTCCA -3' (SEQ ID NO: 10)

RW96: 5' - GGCATCATCATGGGCACCTTCATCCTCTGCTGGCTGCC

CTTCTTC -3' (SEQ ID NO: 11)

25

RW97: 5' - GCAGAAGGGCAGAACAAAGAGCCACGATGAAGAAGGGCA

GCCAGCA -3' (SEQ ID NO: 12)

RW98: 5' - TGGCTGTCATCGGACATCACTTGTTGCACTGCCTCCAT

CCTGCAC -3' (SEQ ID NO: 13)

RW99: 5' - GTAGCGGTCCAGGGCGATGACACAGAGGTGCAGGATGG

30

AGGCAGT -3' (SEQ ID NO: 14)

T604: 5' - CCAGCCGAAGAAGGTGGCAGACTCCA -3' (SEQ ID NO:  
15)

T94: 5' - CTTCTAGGCCTGTACGGAAGTGTTA -3' (SEQ ID NO: 16)

T603: 5' - GCACAGGCTGCGAGTTCTATTCCTT -3' (SEQ ID NO: 17)

35

T605: 5' - CTGGTAATCACACACCCGCTGGCCACCTTTGGTGTGGC

GTCCAAG -3' (SEQ ID NO: 18)



-81-

the art. The cells used to express human SNORF36 receptor were COS-7 and Human embryonic kidney (HEK) 293 cells.

5 COS-7 cells are grown on 150 mm plates in DMEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37°C, 5% CO<sub>2</sub>. Stock plates of COS-7 cells are trypsinized and split 1:6 every 3-4 days.

10 HEK293 cells are grown on 150 mm plates in DMEM with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37°C, 5% CO<sub>2</sub>. Stock plates of 293 cells are trypsinized and split 1:6 every 3-4 days.

15

#### Transient expression

DNA encoding proteins to be studied can be transiently expressed in a variety of mammalian, insect, amphibian and other cell lines by several methods, such as, calcium phosphate-mediated, DEAE-dextran mediated, liposomal-mediated, viral-mediated, electroporation-mediated and microinjection delivery. Each of these methods may require optimization of assorted experimental parameters depending on the DNA, cell line, and the type of assay to be subsequently employed.

25

A typical protocol for the DEAE-dextran method as applied to Cos-7 and HEK293 cells is described as follows. Cells to be used for transfection are split 24 hours prior to the transfection to provide flasks which are 70-80% confluent at the time of transfection. Briefly, 8 µg of receptor DNA plus 8 µg of any additional DNA needed (e.g. G<sub>α</sub> protein expression vector, reporter construct, antibiotic resistance marker, mock vector, etc.) are added to 9 ml of complete DMEM plus DEAE-dextran mixture (10 mg/ml in PBS). Cells plated into

30  
35

-82-

a T225 flask (sub-confluent) are washed once with PBS and the DNA mixture is added to each flask. The cells are allowed to incubate for 30 minutes at 37°C, 5% CO<sub>2</sub>. Following the incubation, 36 ml of complete DMEM with 80 µM chloroquine is added to each flask and allowed to incubate an additional 3 hours. The medium is then aspirated and 24 ml of complete medium containing 10% DMSO for exactly 2 minutes and then aspirated. The cells are then washed 2 times with PBS and 30 ml of complete DMEM added to each flask. The cells are then allowed to incubate over night. The next day the cells are harvested by trypsinization and reseeded into 96 well plates.

#### Stable expression

Heterologous DNA can be stably incorporated into host cells, causing the cell to perpetually express a foreign protein. Methods for the delivery of the DNA into the cell are similar to those described above for transient expression but require the co-transfection of an ancillary gene to confer drug resistance on the targeted host cell. The ensuing drug resistance can be exploited to select and maintain cells that have taken up the DNA. An assortment of resistance genes are available including but not restricted to neomycin, kanamycin, and hygromycin. For purposes of studies concerning the receptor of this invention, stable expression of a heterologous receptor protein is typically carried out in, mammalian cells including but not necessarily restricted to, CHO, HEK293, LM(tk-), etc. In addition native cell lines that naturally carry and express the nucleic acid sequences for the receptor may be used without the need to engineer the receptor complement.

#### Functional assays

Cells expressing the receptor DNA of this invention may be used to screen for ligands to said receptor using functional



-83-

assays. Once a ligand is identified the same assays may be used to identify agonists or antagonists of the receptor that may be employed for a variety of therapeutic purposes. It is well known to those in the art that the over-expression of a GPCR can result in the constitutive activation of intracellular signaling pathways. In the same manner, over-expression of the SNORF36a receptor in any cell line as described above, can result in the activation of the functional responses described below, and any of the assays herein described can be used to screen for agonist, partial agonist, inverse agonist and antagonist ligands of the SNORF36 receptor.

A wide spectrum of assays can be employed to screen for the presence of SNORF36 receptor ligands. These assays range from traditional measurements of total inositol phosphate accumulation, cAMP levels, intracellular calcium mobilization, and potassium currents, for example; to systems measuring these same second messengers but which have been modified or adapted to be of higher throughput, more generic and more sensitive; to cell based assays reporting more general cellular events resulting from receptor activation such as metabolic changes, differentiation, cell division/proliferation. Description of several such assays follow.

#### Cyclic AMP (cAMP) assay

The receptor-mediated stimulation or inhibition of cyclic AMP (cAMP) formation may be assayed in cells expressing the receptors. Cells are plated in 96-well plates or other vessels and preincubated in a buffer such as HEPES buffered saline (NaCl (150 mM), CaCl<sub>2</sub> (1 mM), KCl (5 mM), glucose (10 mM)) supplemented with a phosphodiesterase inhibitor such as 5mM theophylline, with or without protease inhibitor cocktail (For example, a typical inhibitor cocktail contains 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 µg/ml phosphoramidon.)

-84-

for 20 min at 37°C, in 5% CO<sub>2</sub>. Test compounds are added with or without 10mM forskolin and incubated for an additional 10 min at 37°C. The medium is then aspirated and the reaction stopped by the addition of 100 mM HCl or other methods. The plates are stored at 4°C for 15 min, and the cAMP content in the stopping solution is measured by radioimmunoassay. Radioactivity may be quantified using a gamma counter equipped with data reduction software. Specific modifications may be performed to optimize the assay for the receptor or to alter the detection method of cAMP.

#### Arachidonic acid release assay

Cells expressing the receptor are seeded into 96 well plates or other vessels and grown for 3 days in medium with supplements. <sup>3</sup>H-arachidonic acid (specific activity = 0.75 µCi/ml) is delivered as a 100 µL aliquot to each well and samples are incubated at 37° C, 5% CO<sub>2</sub> for 18 hours. The labeled cells are washed three times with medium. The wells are then filled with medium and the assay is initiated with the addition of test compounds or buffer in a total volume of 250 µL. Cells are incubated for 30 min at 37°C, 5% CO<sub>2</sub>. Supernatants are transferred to a microtiter plate and evaporated to dryness at 75°C in a vacuum oven. Samples are then dissolved and resuspended in 25 µL distilled water. Scintillant (300 µL) is added to each well and samples are counted for <sup>3</sup>H in a Trilux plate reader. Data are analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

#### Intracellular calcium mobilization assays

The intracellular free calcium concentration may be measured by microspectrofluorimetry using the fluorescent indicator dye Fura-2/AM (Bush et al, 1991). Cells expressing the receptor are seeded onto a 35 mm culture dish containing a glass coverslip insert and allowed to adhere overnight. Cells are then washed with HBS and loaded with 100 µL of

-85-

Fura-2/AM (10  $\mu$ M) for 20 to 40 min. After washing with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10 to 20 min. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope and fluorescence emission is determined at 510 nM with excitation wavelengths alternating between 340 nM and 380 nM. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques.

In another method, the measurement of intracellular calcium can also be performed on a 96-well (or higher) format and with alternative calcium-sensitive indicators, preferred examples of these are: aequorin, Fluo-3, Fluo-4, Fluo-5, Calcium Green-1, Oregon Green, and 488 BAPTA. After activation of the receptors with agonist ligands the emission elicited by the change of intracellular calcium concentration can be measured by a luminometer, or a fluorescence imager; a preferred example of this is the fluorescence imager plate reader (FLIPR).

Cells expressing the receptor of interest are plated into clear, flat-bottom, black-wall 96-well plates (Costar) at a density of 30,000-80,000 cells per well and allowed to incubate over night at 5% CO<sub>2</sub>, 37°C. The growth medium is aspirated and 100  $\mu$ l of dye loading medium is added to each well. The loading medium contains: Hank's BSS (without phenol red) (Gibco), 20 mM HEPES (Sigma), 0.1% BSA (Sigma), dye/pluronic acid mixture (e.g. 1 mM Fluo-3, AM (Molecular Probes), 10% pluronic acid (Molecular Probes); (mixed immediately before use), and 2.5 mM probenecid (Sigma) (prepared fresh)). The cells are allowed to incubate for about 1 hour at 5% CO<sub>2</sub>, 37°C.

During the dye loading incubation the compound plate is prepared. The compounds are diluted in wash buffer (Hank's

-86-

BSS without phenol red), 20 mM HEPES, 2.5 mM probenecid to a 3X final concentration and aliquoted into a clear v-bottom plate (Nunc). Following the incubation the cells are washed to remove the excess dye. A Denley plate washer is used to gently wash the cells 4 times and leave a 100  $\mu$ l final volume of wash buffer in each well. The cell plate is placed in the center tray and the compound plate is placed in the right tray of the FLIPR. The FLIPR software is setup for the experiment, the experiment is run and the data are collected. The data are then analyzed using an excel spreadsheet program.

Antagonist ligands are identified by the inhibition of the signal elicited by agonist ligands.

The intracellular free calcium ( $\text{Ca}^{2+}$ ) concentration may be measured by the Fluorescence Imager Plate Reader (FLIPR<sup>TM</sup>).

Cells transfected with appropriate DNA as described earlier were plated into clear, flat-bottom, black-wall 96-well plates (Costar) at a density of 80,000-150,000 cells per well and allowed to incubate for 24 hr at 5%  $\text{CO}_2$ , 37°C. Whenever necessary, the cells were placed in a waterbath maintained at 37°C and were exposed to a lamp light (50W) placed at a distance of approximately 30 cm, 90 to 120 min before the imaging. The growth medium was aspirated and 100  $\mu$ l of loading medium containing fluo-3 dye was added to each well 60 min before the imaging. The loading medium contained: Hank's BSS (without phenol red) (Gibco), 20 mM HEPES (Sigma), 0.1 or 1% BSA (Sigma), dye/pluronic acid mixture (e.g. 1 mM Flou-3, AM (Molecular Probes) and 10% pluronic acid (Molecular Probes) mixed immediately before use), and 2.5 mM probenecid (Sigma) (prepared fresh). The cells were allowed to incubate for about 1 hour at 5%  $\text{CO}_2$ , 37°C.

Before or during the incubation of cells with the dye-loading

-87-

medium, the test compound plate was prepared. Since retinoids are chemically unstable and can undergo rapid photoisomerization (Hu et al., 1994), care was taken to weigh, dissolve and prepare appropriate concentrations of the test compounds under photographic darkroom lights. The test compounds were diluted in wash buffer (Hank's BSS (without phenol red), 20 mM HEPES, 2.5 mM probenecid) to a 4X final concentration and aliquoted into a clear v-bottom plate (Nunc). The test compounds were protected from light till their addition to cells. Following the incubation with the dye-loading medium, the cells were washed to remove the excess dye. A Denley plate washer was used to gently wash the cells 4 times and leave a 100  $\mu$ l final volume of wash buffer in each well. The cell plate was placed in the center tray and the test compound plate was placed in the right tray of the FLIPR. The FLIPR software was setup for the experiment, the experiment was run and the data were collected. For the agonist experiment, the sampling rate in the FLIPR was every 1 sec for the first minute and every 2 sec for the next two minutes. In this paradigm, the test compound was added after recording base line for the first 10 sec. For the antagonist experiment, the sampling rate in the FLIPR was every 1 sec for the first minute, every 6 sec for the next 5 minutes, every 1 sec for the next one minute followed by every 2 sec next two minutes. To evaluate the antagonistic activity, the test compound was added to the cells 10 sec after commencing the recording and the agonist was added at 310 sec of the recording. Baseline subtraction and negative control corrections were performed on the traces. The collected data were then analyzed using an Excel spreadsheet program.

#### Inositol phosphate assay

Human SNORF36a receptor-mediated activation of the inositol phosphate (IP) second messenger pathways was assessed by radiometric measurement of IP products.

Cells were plated at a density of 70,000 cells per well and allowed to incubate for 24 hours. The cells were then labeled with 0.5  $\mu$ Ci [ $^3$ H]myo-inositol overnight at 37°C, 5% CO<sub>2</sub>. Immediately before the assay, the medium was removed and replaced with 180  $\mu$ L of Phosphate-Buffered Saline (PBS) containing 10 mM LiCl. The plates were then incubated for 20 min at 37°C, 5% CO<sub>2</sub>. Following the incubation, the cells were challenged with agonist (20  $\mu$ l/well; 10x concentration) for 30 min at 37°C and were simultaneously either exposed or not to a lamp light (50W) placed at approximately 30 cm distance from the cells. The challenge was terminated by the addition

-89-

of 100  $\mu$ L of 5% v/v trichloroacetic acid, followed by incubation at 4°C for greater than 30 minutes. Total IPs were isolated from the lysate by ion exchange chromatography. Briefly, the lysed contents of the wells were transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates were prepared adding 100  $\mu$ L of Dowex AG1-X8 suspension (50% v/v, water: resin) to each well. The filter plates were placed on a vacuum manifold to wash or elute the resin bed. Each well was first washed 2 times with 200  $\mu$ L of 5 mM myo-inositol. Total [ $^3$ H]inositol phosphates were eluted with 75  $\mu$ L of 1.2M ammonium formate/0.1M formic acid solution into 96-well plates. 200  $\mu$ L of scintillation cocktail was added to each well, and the radioactivity was determined by liquid scintillation counting.

#### GTP $\gamma$ S functional assay

Membranes from cells expressing the receptor are suspended in assay buffer (e.g., 50 mM Tris, 100 mM NaCl, 5 mM MgCl<sub>2</sub>, 10  $\mu$ M GDP, pH 7.4) with or without protease inhibitors (e.g., 0.1% bacitracin). Membranes are incubated on ice for 20 minutes, transferred to a 96-well Millipore microtiter GF/C filter plate and mixed with GTP $\gamma$ <sup>35</sup>S (e.g., 250,000 cpm/sample, specific activity ~1000 Ci/mmol) plus or minus unlabeled GTP $\gamma$ S (final concentration = 100  $\mu$ M). Final membrane protein concentration  $\approx$  90  $\mu$ g/ml. Samples are incubated in the presence or absence of test compounds for 30 min. at room temperature, then filtered on a Millipore vacuum manifold and washed three times with cold (4°C) assay buffer. Samples collected in the filter plate are treated with scintillant and counted for <sup>35</sup>S in a Trilux (Wallac) liquid scintillation counter. It is expected that optimal results are obtained when the receptor membrane preparation is derived from an appropriately engineered heterologous expression system, i.e., an expression system resulting in high levels of expression of the receptor and/or expressing

-90-

G-proteins having high turnover rates (for the exchange of GDP for GTP). GTPyS assays are well-known to those skilled in the art, and it is contemplated that variations on the method described above, such as are described by Tian et al. (1994) or Lazareno and Birdsall (1993), may be used.

#### Microphysiometric assay

Because cellular metabolism is intricately involved in a broad range of cellular events (including receptor activation of multiple messenger pathways), the use of microphysiometric measurements of cell metabolism can in principle provide a generic assay of cellular activity arising from the activation of any orphan receptor regardless of the specifics of the receptor's signaling pathway.

General guidelines for transient receptor expression, cell preparation and microphysiometric recording are described elsewhere (Salon, J.A. and Owicki, J.A., 1996). Typically cells expressing receptors are harvested and seeded at  $3 \times 10^5$  cells per microphysiometer capsule in complete media 24 hours prior to an experiment. The media is replaced with serum free media 16 hours prior to recording to minimize non-specific metabolic stimulation by assorted and ill-defined serum factors. On the day of the experiment the cell capsules are transferred to the microphysiometer and allowed to equilibrate in recording media (low buffer RPMI 1640, no bicarbonate, no serum (Molecular Devices Corporation, Sunnyvale, CA) containing 0.1% fatty acid free BSA), during which a baseline measurement of basal metabolic activity is established.

A standard recording protocol specifies a 100  $\mu$ l/min flow rate, with a 2 min total pump cycle which includes a 30 sec flow interruption during which the acidification rate measurement is taken. Ligand challenges involve a 1 min 20 sec exposure to the sample just prior to the first post



-91-

challenge rate measurement being taken, followed by two additional pump cycles for a total of 5 min 20 sec sample exposure. Typically, drugs in a primary screen are presented to the cells at 10  $\mu$ M final concentration.

Follow up experiments to examine dose-dependency of active compounds are then done by sequentially challenging the cells with a drug concentration range that exceeds the amount needed to generate responses ranging from threshold to maximal levels. Ligand samples are then washed out and the acidification rates reported are expressed as a percentage increase of the peak response over the baseline rate observed just prior to challenge.

#### MAP kinase assay

MAP kinase (mitogen activated kinase) may be monitored to evaluate receptor activation. MAP kinase is activated by multiple pathways in the cell. A primary mode of activation involves the ras/raf/MEK/MAP kinase pathway. Growth factor (tyrosine kinase) receptors feed into this pathway via SHC/Grb-2/SOS/ras. Gi coupled receptors are also known to activate ras and subsequently produce an activation of MAP kinase. Receptors that activate phospholipase C (such as Gq/G11-coupled) produce diacylglycerol (DAG) as a consequence of phosphatidyl inositol hydrolysis. DAG activates protein kinase C which in turn phosphorylates MAP kinase.

MAP kinase activation can be detected by several approaches. One approach is based on an evaluation of the phosphorylation state, either unphosphorylated (inactive) or phosphorylated (active). The phosphorylated protein has a slower mobility in SDS-PAGE and can therefore be compared with the unstimulated protein using Western blotting. Alternatively, antibodies specific for the phosphorylated protein are available (New England Biolabs) which can be used to detect

-92-

an increase in the phosphorylated kinase. In either method, cells are stimulated with the test compound and then extracted with Laemmli buffer. The soluble fraction is applied to an SDS-PAGE gel and proteins are transferred electrophoretically to nitrocellulose or Immobilon. Immunoreactive bands are detected by standard Western blotting technique. Visible or chemiluminescent signals are recorded on film and may be quantified by densitometry.

Another approach is based on evaluation of the MAP kinase activity via a phosphorylation assay. Cells are stimulated with the test compound and a soluble extract is prepared. The extract is incubated at 30°C for 10 min with gamma-<sup>32</sup>P-ATP, an ATP regenerating system, and a specific substrate for MAP kinase such as phosphorylated heat and acid stable protein regulated by insulin, or PHAS-I. The reaction is terminated by the addition of H<sub>3</sub>PO<sub>4</sub> and samples are transferred to ice. An aliquot is spotted onto Whatman P81 chromatography paper, which retains the phosphorylated protein. The chromatography paper is washed and counted for <sup>32</sup>P in a liquid scintillation counter. Alternatively, the cell extract is incubated with gamma-<sup>32</sup>P-ATP, an ATP regenerating system, and biotinylated myelin basic protein bound by streptavidin to a filter support. The myelin basic protein is a substrate for activated MAP kinase. The phosphorylation reaction is carried out for 10 min at 30°C. The extract can then be aspirated through the filter, which retains the phosphorylated myelin basic protein. The filter is washed and counted for <sup>32</sup>P by liquid scintillation counting.

#### Cell proliferation assay

Receptor activation of the orphan receptor may lead to a mitogenic or proliferative response which can be monitored via <sup>3</sup>H-thymidine uptake. When cultured cells are incubated with <sup>3</sup>H-thymidine, the thymidine translocates into the nuclei

5

20

## 25

30

35

-94-

$G_{\alpha 16}$  or a chimeric  $G_{\alpha}$  subunit such as  $G_{\alpha qz}$ , a GPCR, which might normally prefer to couple through a specific signaling pathway (e.g.,  $G_s$ ,  $G_i$ ,  $G_q$ ,  $G_o$ , etc.), can be made to couple through the pathway defined by the promiscuous  $G_{\alpha}$  subunit and upon agonist activation produce the second messenger associated with that subunit's pathway. In the case of  $G_{\alpha 15}$ ,  $G_{\alpha 16}$  and/or  $G_{\alpha qz}$  this would involve activation of the  $G_q$  pathway and production of the second messenger  $IP_3$ . Through the use of similar strategies and tools, it is possible to bias receptor signaling through pathways producing other second messengers such as  $Ca^{++}$ , cAMP, and  $K^+$  currents, for example (Milligan, 1999).

It follows that the promiscuous interaction of the exogenously supplied  $G_{\alpha}$  subunit with the orphan receptor alleviates the need to carry out a different assay for each possible signaling pathway and increases the chances of detecting a functional signal upon receptor activation.

#### Methods for recording currents in *Xenopus* oocytes

Oocytes are harvested from *Xenopus laevis* and injected with mRNA transcripts as previously described (Quick and Lester, 1994; Smith et al., 1997). The test receptor of this invention and  $G_{\alpha}$  subunit RNA transcripts are synthesized using the T7 polymerase ("Message Machine," Ambion) from linearized plasmids or PCR products containing the complete coding region of the genes. Oocytes are injected with 10 ng synthetic receptor RNA and incubated for 3-8 days at 17 degrees. Three to eight hours prior to recording, oocytes are injected with 500 pg promiscuous  $G_{\alpha}$  subunits mRNA in order to observe coupling to  $Ca^{++}$  activated  $Cl^-$  currents. Dual electrode voltage clamp (Axon Instruments Inc.) is performed using 3 M KCl-filled glass microelectrodes having resistances of 1-2 MOhm. Unless otherwise specified, oocytes are voltage clamped at a holding potential of -80 mV. During

-95-

recordings, oocytes are bathed in continuously flowing (1-3 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, and 5 mM HEPES, pH 7.5 (ND96). Drugs are applied either by local perfusion from a 10 µl glass capillary tube fixed at a distance of 0.5 mm from the oocyte, or by switching from a series of gravity fed perfusion lines.

Other oocytes may be injected with a mixture of receptor mRNAs and synthetic mRNA encoding the genes for G-protein-activated inward rectifier channels (GIRK1 and GIRK4, U.S. Patent Nos. 5,734,021 and 5,728,535 or GIRK1 and GIRK2) or any other appropriate combinations (see, e.g., Inanobe et al., 1999). Genes encoding G-protein inwardly rectifying K<sup>+</sup> (GIRK) channels 1, 2 and 4 (GIRK1, GIRK2, and GIRK4) may be obtained by PCR using the published sequences (Kubo et al., 1993; Dascal et al., 1993; Krapivinsky et al., 1995 and 1995b) to derive appropriate 5' and 3' primers. Human heart or brain cDNA may be used as template together with appropriate primers.

Heterologous expression of GPCRs in *Xenopus* oocytes has been widely used to determine the identity of signaling pathways activated by agonist stimulation (Gundersen et al., 1983; Takahashi et al., 1987). Activation of the phospholipase C (PLC) pathway is assayed by applying test compound in ND96 solution to oocytes previously injected with mRNA for the mammalian orphan receptor (with or without promiscuous G proteins) and observing inward currents at a holding potential of -80 mV. The appearance of currents that reverse at -25 mV and display other properties of the Ca<sup>2+</sup>-activated Cl<sup>-</sup> (chloride) channel is indicative of mammalian receptor-activation of PLC and release of IP3 and intracellular Ca<sup>2+</sup>. Such activity is exhibited by GPCRs that couple to G<sub>q</sub> or G<sub>11</sub>.

Measurement of inwardly rectifying K<sup>+</sup> (potassium) channel

-96-

(GIRK) activity may be monitored in oocytes that have been co-injected with mRNAs encoding the mammalian orphan receptor plus GIRK subunits. GIRK gene products co-assemble to form a G-protein activated potassium channel known to be activated (i.e., stimulated) by a number of GPCRs that couple to  $G_i$  or  $G_o$  (Kubo et al., 1993; Dascal et al., 1993). Oocytes expressing the mammalian orphan receptor plus the GIRK subunits are tested for test compound responsivity by measuring  $K^+$  currents in elevated  $K^+$  solution containing 49 mM  $K^+$ .

In the present invention, oocytes were harvested from *Xenopus laevis* and injected with mRNA transcripts as previously described (Quick and Lester, 1994; Smith et al., 1997). SNORF36a RNA transcripts were synthesized using the T7 polymerase ("Message Machine", Ambion) from the plasmid B0109 linearized with NotI. Oocytes were injected with 5-25 ng synthetic RNA and incubated for 3-8 days at 17°C. Dual electrode voltage clamp (Axon Instruments Inc.) was performed using 3 M KCl-filled glass microelectrodes having resistances of 1-2 MOhm. Unless otherwise specified, oocytes were voltage clamped at a holding potential of -80 mV. During recordings, oocytes were bathed in continuously flowing (1-3 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM  $CaCl_2$ , 1 mM  $MgCl_2$ , 5 mM HEPES, pH 7.5 (ND96), and the appropriate ligand.

Experiments were carried out under minimal light conditions which required compound weighing, final dilutions and oocyte recordings to be performed under photographic darkroom lights ("Brightlab Junior" safelight). In some cases, the ligands were briefly exposed to room light (5-10 min.) during the weighing procedure. For eliciting physiological responses from oocytes, the light stimulus was a 60 W tungsten lamp set at a distance of 30 cm from the oocyte. Drugs were applied

-97-

either by superfusion, switching from a series of gravity fed perfusion lines, or by local perfusion from a 10  $\mu$ l glass capillary tube fixed at a distance of 0.5 mm from the oocyte. Experiments were carried out at room temperature. All values are expressed as mean  $\pm$  standard error of the mean.

#### Membrane preparations

Cell membranes expressing the receptor protein of this invention are useful for certain types of assays including but not restricted to ligand binding assays, GTP-g-S binding assays, and others. The specifics of preparing such cell membranes may in some cases be determined by the nature of the ensuing assay but typically involve harvesting whole cells and disrupting the cell pellet by sonication in ice cold buffer (e.g. 20 mM Tris HCl, mM EDTA, pH 7.4 at 4° C). The resulting crude cell lysate is cleared of cell debris by low speed centrifugation at 200xg for 5 min at 4° C. The cleared supernatant is then centrifuged at 40,000xg for 20 min at 4° C, and the resulting membrane pellet is washed by suspending in ice cold buffer and repeating the high speed centrifugation step. The final washed membrane pellet is resuspended in assay buffer. Protein concentrations are determined by the method of Bradford (1976) using bovine serum albumin as a standard. The membranes may be used immediately or frozen for later use.

#### Generation of baculovirus

The coding region of DNA encoding the human receptor disclosed herein may be subcloned into pBlueBacIII into existing restriction sites or sites engineered into sequences 5' and 3' to the coding region of the polypeptides. To generate baculovirus, 0.5  $\mu$ g of viral DNA (BaculoGold) and 3  $\mu$ g of DNA construct encoding a polypeptide may be co-transfected into  $2 \times 10^6$  *Spodoptera frugiperda* insect Sf9 cells by the calcium phosphate co-precipitation method, as

outlined by Pharmingen (in "Baculovirus Expression Vector System: Procedures and Methods Manual"). The cells then are incubated for 5 days at 27°C.

5 The supernatant of the co-transfection plate may be collected by centrifugation and the recombinant virus plaque purified. The procedure to infect cells with virus, to prepare stocks of virus and to titer the virus stocks are as described in Pharmingen's manual.

10

Localization of mRNA coding for human and rat SNORF36

Quantitative RT-PCR using a fluorogenic probe with real time detection: Quantitative RT-PCR using fluorogenic probes and  
15 a panel of mRNA extracted from human and total RNA extracted from rat tissue was used to characterize the localization of rat and human SNORF36.

This assay utilizes two oligonucleotides for conventional PCR  
20 amplification and a third specific oligonucleotide probe that is labeled with a reporter at the 5' end and a quencher at the 3' end of the oligonucleotide. In the instant invention, FAM (6-carboxyfluorescein) and JOE (6 carboxy-4,5-dichloro-2,7-dimethoxyfluorescein) were the two reporters that were  
25 utilized and TAMRA (6-carboxy-4,7,2,7'-tetramethylrhodamine) was the quencher. As amplification progresses, the labeled oligonucleotide probe hybridizes to the gene sequence between the two oligonucleotides used for amplification. The  
30 nuclease activity of Taq, or rTth thermostable DNA polymerases is utilized to cleave the labeled probe. This separates the quencher from the reporter and generates a fluorescent signal that is directly proportional to the amount of amplicon generated. This labeled probe confers a  
35 high degree of specificity. Non-specific amplification is not detected as the labeled probe does not hybridize. All experiments were conducted in a PE7700 Sequence Detection



-99-

System (Perkin Elmer, Foster City CA),

Quantitative RT-PCR: For the detection of RNA encoding SNORF36 receptors, quantitative RT-PCR was performed on mRNA  
5 extracted from tissue. Reverse transcription and PCR reactions were carried out in 50 µl volumes using rTth DNA polymerase (Perkin Elmer).

10 Primers for human SNORF36 were designed to amplify the long subtype selectively (SNORF36b) or to a region common to both SNORF36a and SNORF36b. Amounts of RNA encoding the short subtype (SNORF36a) were calculated by subtracting the amount of SNORF36b from the amount of total SNORF36. Primers with the following sequences were used:

15 Human SNORF36 subtype non-selective:

Forward primer:

SNORF36b2-457F

20 5'-GGCTGCGAGTTCTATGCCTT-3' (SEQ ID NO: 36)

Reverse primer

SNORF36b2-547r

25 5'-TTACCAGGTAGCGGTCCAGG-3' (SEQ ID NO: 37)

Fluorogenic oligonucleotide probe:

SNORF36b2-483T

30 5' (6-FAM)-AGCTCTCTTTGGCATTTCCTCCATGATCA-(TAMRA) 3' (SEQ ID NO: 38)

Human SNORF36b (long subtype) selective:

Forward primer:

SNORF36b b sel-256F

35 5'-CTGGGCAACCTGACGGTC-3' (SEQ ID NO: 39)

Using these primer pairs, amplicon length is 90 bp for human  
25 SNORF36-non subtype-selective, 90 bp for human SNORF36b-  
selective, and 73 bp for rat SNORF36. Each human SNORF36 RT-  
PCR reaction contained 50 ng mRNA and each rat SNORF36 RT-PCR  
reaction contained 100 ng total RNA. Oligonucleotide  
concentrations were: 500 nM of forward and reverse primers,  
30 and 200 nM of fluorogenic probe. Concentrations of reagents  
in each reaction were: 300  $\mu$ M each of dGTP; dATP; dCTP; 600  
 $\mu$ M UTP; 3.0mM  $Mn(OAc)_2$  ; 50 mM Bicine; 115 mM potassium  
acetate, 8% glycerol, 5 units rTth DNA polymerase, and 0.5  
units of uracil N-glycosylase. Buffer for RT-PCR reactions  
35 also contained a fluor used as a passive reference (ROX:

-101-

Perkin Elmer proprietary passive reference I). All reagents for RT-PCR (except mRNA and oligonucleotide primers) were obtained from Perkin Elmer (Foster City, CA). Reactions were carried using the following thermal cycler profile: 50°C 2 min., 60°C 30 min., 95°C 5 min., followed by 40 cycles of: 94°C, 20 sec., 62°C 1 min.

Positive controls for PCR reactions consisted of amplification of the target sequence from a plasmid construct when available. Standard curves for quantitation of human SNORF36 were constructed using the SNORF36 gene in a plasmid construct. RNA extracted from whole brain was used to construct a standard curve for rat SNORF36. Negative controls consisted of mRNA blanks, as well as primer and mRNA blanks. To confirm that the mRNA was not contaminated with genomic DNA, PCR reactions were carried out without reverse transcription using Taq DNA polymerase. Integrity of RNA was assessed by amplification of RNA coding for cyclophilin or glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Following reverse transcription and PCR amplification, data was analyzed using Perkin Elmer sequence detection software. The fluorescent signal from each well was normalized using an internal passive reference, and data was fitted a standard curve to obtain relative quantities of SNORF36 mRNA expression.

#### Expression of SNORF36 mRNA in human retina

To determine if SNORF36 mRNA is expressed in retina, RT-PCR was performed on human retina Quick Clone cDNA (Clontech, Palo Alto, CA) using two different primer pairs (BB788, a forward SNORF36 primer, with BB789, a reverse SNORF36 primer; or BB796, a forward SNORF36 primer with BB798, a reverse SNORF36 primer). PCR was performed using the Expand Long Template PCR System (Roche Molecular Biochemicals, Indianapolis, IN) under the following conditions: 94°C hold

Chromosomal localization for the human SNORF36 receptor gene was established using a panel of radiation hybrids prepared by the Stanford Human Genome Center (SHGC) and distributed by Research Genetics, Inc. The "Stanford G3" panel of 83 radiation hybrids was analyzed by PCR using the same primers, probes and thermal cycler profiles as used for localization. 20 ng of DNA was used in each PCR reaction. Data was submitted to the RH Server (SHGC) which linked the SNORF36 gene sequences to specific markers. NCBI LocusLink and NCBI GeneMap '99 were used to further analyze the data.

## RESULTS AND DISCUSSION

### Identification of a fragment of the human SNORF36 receptor

5 A human placental genomic library was screened, under reduced stringency conditions, with oligonucleotide probes directed to the third, fifth and sixth transmembrane regions of the human serotonin 5-HT<sub>1D</sub> receptor. Positively-hybridizing clones were isolated, plaque-purified, characterized by  
10 Southern blot analysis, and sequenced. One clone, hp10b, contained a 2.3 kb *Hind*III/*Xba*I fragment which hybridized with the human 5-HT<sub>1D</sub>-derived oligonucleotide probes and was subsequently subcloned into a pUC vector. This clone, called K39, was a partial gene fragment, encoding TMII and MIII and  
15 a possible downstream intron of a putative novel GPCR.

### Isolation of a full-length human SNORF36 receptor

In order to obtain additional sequence for this receptor, a human hippocampal cDNA library was screened by PCR using  
20 primers directed against K39. One positive pool was successfully subdivided until a single clone, TL252, was isolated. DNA sequencing of this clone revealed that it contained TMI through TMVII but was lacking the NH<sub>2</sub> and COOH termini. The remaining sequence, including the initiating methionine and the stop codon were obtained by 5' and 3' RACE  
25 performed on human hippocampal cDNA. The human hippocampal cDNA library was re-screened and two positive clones were identified, 260-13-1 and 143-33-3, which contained TMI through the stop codon. These two clones both had an 11  
30 amino acid insert in the first intracellular loop that was not present in TL252.

A full-length cDNA clone for SNORF36a (without the 11 amino acid insert) was obtained by ligating together a 1021 bp  
35 *Bam*HI/*Bgl*II fragment from a hippocampal cDNA PCR product along

-104-

with a 1180 bp *BglII/EcoRI* fragment from the human hippocampal library pool 260.13.1 into a *BamHI/EcoRI*-cut pcDNA3.1. This construct, BO108, had a single nucleotide mutation which was corrected by site-directed mutagenesis. The corrected construct, BO109, was renamed pcDNA3.1-hSNORF36a-f. Analysis of 9 independent PCR products from human hippocampal cDNA revealed the presence of an allelic variation. Nucleotide 39 (Figures 1A-1C and 3A-3C) is either an adenine or a guanine. A full-length clone for SNORF36b (with the 11 amino acid insert) was obtained by ligating together a 1130 bp *NspI/HindIII* fragment from a PCR of BO108 along with a 390 bp *BamHI/NspI* fragment from one of the PCR products described above into a *BamHI/HindIII*-cut pcDNA3.1. The resulting SNORF36b construct, BO110, was renamed pcDNA3.1-hSNORF36b-f.

The largest open reading frame in SNORF36a is 1434 nucleotides, which is predicted to encode a protein of 478 amino acids. Using a downstream methionine results in an open frame of 1377 nucleotides and is predicted to encode a protein of 459 amino acids. The nucleotide and amino acid sequences of SNORF36a are shown in Figures 1A-1C and 2A-2B, respectively. The largest open reading frame in SNORF36b is 1467 nucleotides, which is predicted to encode a protein of 489 amino acids. Using a downstream methionine results in an open frame of 1410 nucleotides and is predicted to encode a protein of 470 amino acids. The nucleotide and amino acid sequences of SNORF36b are shown in Figures 3A-3C and 4A-4C respectively. Hydropathy analysis of both SNORF36a and SNORF36b protein are consistent with a putative topography of seven transmembrane domains, indicative of the G protein-coupled receptor family.

A comparison of nucleotide and peptide sequences of human SNORF36a and SNORF36b with sequences contained in the Genbank, EMBL, and SwissProtPlus databases reveals that the



-106-

shares 86% nucleotide and 88% amino acid identities with the human SNORF36 (Figures 7 and 8). KO56 was renamed pEXJ-T3T7-rSNORF36p. There were no sequences in the Genbank databases (Genembl, STS, EST, GSS, or SwissProt) that were identical to rat SNORF36.

#### Isolation of a full-length rat SNORF36 receptor

In order to obtain a full-length cDNA for rat SNORF36, a rat spinal cord cDNA library was screened by PCR using primers directed against the rat SNORF36 fragment, KO56. One positive pool was successfully subdivided until a single clone, B0132, was isolated. DNA sequencing of this clone revealed that it contained an open reading frame of 1422 nucleotides, 148 nucleotides of 5'UT and approximately 1000 nucleotides of 3'UT. The construct B0132, in the expression vector pEXJ.T7, was renamed pEXJ.T7-rSNORF36-f.

The largest open reading frame in the rat SNORF36 construct B0132 is 1422 nucleotides, which is predicted to encode a protein of 474 amino acids. The nucleotide and amino acid sequences of rat SNORF36 are shown in Figures 9A-9C and 10A-10C, respectively. Hydropathy analysis of the rat SNORF36 protein are consistent with a putative topography of seven transmembrane domains, indicative of the G protein-coupled receptor family.

Rat SNORF36 shares 81% nucleotide identity and 79% amino acid identity with human SNORF36a (Figures 11A-11D and 12A-12B, respectively). A comparison of nucleotide and peptide sequences of rat SNORF36 with sequences contained in the Genbank, EMBL, and SwissProtPlus databases reveals that the amino acid sequences of these clones are most related to the *Xenopus* melanopsin receptor (46% identity), giant octopus rhodopsin and giant scallop rhodopsin (35% identities), cuttlefish rhodopsin (34% identity), Japanese flying squid



-107-

and mouse peropsin (33% identities), human RGR (32% identity), human and bovine rhodopsin (28% identities) and human encephalopsin (27% identity). There were no sequences in the Genbank databases (Genembl, STS, EST, GSS, or SwissProt) that were identical to rat SNORF36.

Rat SNORF36 has six potential protein kinase C (PKC) phosphorylation motifs at serine 183 in the second intracellular loop, threonine 265 in the third intracellular loop, and at serines 381 and 385, and threonines 460 and 463 in the carboxy-terminal tail. There are also three potential N-linked glycosylation sites at asparagines 31 and 35 in the amino terminal tail and at asparagine 88 in the first transmembrane domain. There is one cAMP phosphorylation site at threonine 187 in the fourth transmembrane domain, and three potential casein kinase II phosphorylation sites at serines 411 and 452 and threonine 418 in the carboxy-terminal tail.

#### Ca<sup>2+</sup> Mobilization and Phosphoinositide Hydrolysis in SNORF36a-expressing Cells

Since SNORF36a is similar to invertebrate opsins, it was hypothesized that it may couple to G<sub>q</sub> G-protein and may induce phosphoinositide hydrolysis followed by release of intracellular Ca<sup>2+</sup>. Therefore, Ca<sup>2+</sup> mobilization and inositol phosphate (IP) accumulation were evaluated in hSNORF36a-transfected Cos-7 cells. Exposure to laser light of hSNORF36a-transfected Cos-7 cells, kept in dark, in the FLIPR resulted in Ca<sup>2+</sup> mobilization in the absence of any exogenous ligand (Figure 13A). This observation suggested that an endogenous chromophore, probably a retinal isomer, was already attached to the hSNORF36a receptor, and exposure to laser light resulted in photoisomerization of the ligand and activation of the receptor. Since this situation did not

-108-

allow us to study the effect of an exogenously added ligand, we 'photobleached' the hSNORF36a- and vector-transfected Cos-7 cells by exposing them to light for 90-120 minutes prior to the experiment. Photobleaching is a phenomenon where exposure to light results in photoisomerization of the endogenous ligand, eventually leading to release of the ligand and formation of an opsin with an empty binding pocket. This technique is used widely to dissociate the endogenous ligand from rhodopsin. Upon photobleaching, the  $\text{Ca}^{2+}$  mobilization response to laser light in the hSNORF36a-transfected Cos-7 cells was either reduced or completely abolished (Figure 13B), implying dissociation of the endogenous ligand. Therefore, photobleaching was used for all subsequent experiments unless indicated otherwise.

Opsins can be activated by several retinal analogues, including all-trans-retinal (Surya and Knox, 1998) which is a 'natural' agonist formed upon photoisomerization of 11-cis-retinal. Upon addition of retinal analogues to photobleached SNORF36a-transfected Cos-7 cells, significant  $\text{Ca}^{2+}$  mobilization was observed (Figures 14A, 15A). The rank order of potency was 9-cis-retinal ( $\text{EC}_{50}$ :152nM) > all-trans-retinal ( $\text{EC}_{50}$ :263 nM) > 13-cis-retinal ( $\text{EC}_{50}$ :477nM) (See Figure 15A). A similar response to all-trans-retinal was seen in photobleached SNORF36a-transfected HEK293 cells (Figure 15B). In contrast, retinals did not change intracellular  $\text{Ca}^{2+}$  levels in the vector-transfected Cos-7 cells (Figure 14B). Collectively, these results indicate that SNORF36a is an opsin which can be activated by retinals. To confirm these results further, we examined the effect of SNORF36a on IP formation. Concentration-dependent increase in  $^3\text{H}$ -IP accumulation was seen upon all-trans-retinal addition (Figure 16A) in SNORF36a-transfected Cos-7 cells exposed to light but not in vector-transfected cells (Figure 16A). In contrast, in SNORF36a-transfected Cos-7 cells not exposed to light,

-109-

all-trans-retinal did not change  $^3\text{H}$ -IP levels, indicating that light may be required either for dissociation of endogenous ligand or activation of the receptor (Figure 16B). Collectively, these results suggest that activation of hSNORF36a can induce phosphoinositide hydrolysis which may be upstream of intracellular  $\text{Ca}^{2+}$  mobilization. Finally, we tested the potential antagonism by  $\beta$ -ionone which is structurally similar to retinals.  $\beta$ -ionone, at  $10\mu\text{M}$  concentration, significantly antagonized responses to all-trans-retinal and 9-cis-retinal in hSNORF36a-transfected Cos-7 cells (Figures 17A and 17B). The antagonism by  $\beta$ -ionone appeared to be insurmountable (Figures 17A and 17B). This may be due to the intrinsic characteristics of  $\beta$ -ionone-hSNORF36a interaction. Alternatively, it may represent the hemi-equilibrium conditions achieved in the assay. Nonetheless, these results confirm that retinal-like structure can bind to this receptor. These results are consistent with the notion that SNORF36a is an opsin coupled to  $\text{Ca}^{2+}$  mobilization and phosphoinositide hydrolysis, most likely via  $\text{G}_q$  G-protein, and is activated by retinals.

#### Photic Responses From Oocytes Expressing SNORF36a

The experiments with mammalian cells suggest that SNORF36a, stimulated by the presence of retinoids, can activate a second messenger response that includes release of inositol triphosphates and intracellular free  $\text{Ca}^{++}$ . Heterologous expression in *Xenopus* oocytes has been widely used to study the ligand binding and functional activity of a variety of G-protein coupled receptors (Gundersen et al., 1983; Takahashi et al., 1987), including rhodopsin (Khorana et al., 1988). Activation of the PLC pathway in oocytes leads to stimulation of the endogenous  $\text{Ca}^{++}$ -activated  $\text{Cl}^-$  current. We sought to determine if oocytes expressing SNORF36a could be stimulated by retinoids, and if sensitivity to retinoids was dependent upon activation by light.



35

-111-

not respond to light (Figure 18C; n = 10 oocytes).

Detection of RNA coding for human SNORF36 receptors

mRNA was isolated from multiple tissues (listed in Table 1) and assayed as described.

Human SNORF36

Quantitative RT-PCR using a fluorogenic probe demonstrated mRNA encoding human SNORF36 to be localized in highest abundance in CNS tissue. All CNS tissues assayed demonstrate measurable levels of SNORF36 RNA.

Highest levels are found in the caudate-putamen, amygdala and hippocampus. Localization of high levels of SNORF36 RNA to the caudate-putamen indicates a role in modulation of dopaminergic function, or the modulation of extrapyramidal motor systems. High levels of SNORF36 RNA in the hippocampal formation and amygdala support the hypothesis that SNORF36 is involved in the modulation of learning and memory. It may also have a role in the regulation of fear, mood, and may provide a target for the treatment of depression, anxiety, phobias and mood disorders. Other regions of the CNS containing SNORF36 RNA include the spinal cord and thalamus, implying an important role in sensory transmission or modulation (including nociception). SNORF36 is also expressed in lower levels in the substantia nigra, hypothalamus, and cerebellum. The broad distribution of SNORF36 RNA throughout the CNS implies a modulatory role in multiple systems within the CNS.

Fetal brain, although expressing SNORF36 mRNA does so in much lower levels than that found in the adult. In fetal brain, SNORF36 RNA is barely detectable. There is a 70-fold difference in mRNA levels between fetal and adult brain. It is not known at this time if the developmental regulation

In summary, the distribution of human SNORF36 mRNA implies broad regulatory function in the CNS, most notably in modulation of extrapyramidal motor systems, modulation of the limbic system, and sensory transmission. Its presence, albeit at lower levels, in peripheral tissues implies a broad regulatory role in multiple organ systems. The predominance of SNORF36a in most tissue indicates that functionally, it is the dominant subtype.

-113-

Rat SNORF36

As with the human SNORF36 receptor mRNA, all central nervous system structures assayed contain rat SNORF36 RNA. However, the highest levels of rat SNORF36 RNA are found in the retina (Table 2). The retina contains 5-fold more RNA encoding SNORF36 than any other tissue. The high levels found in the retina imply a function in vision or photoentrainment. The hypothesis that rat SNORF36 plays a role in circadian rhythm is supported by the fact that substantial levels of SNORF36 RNA are also expressed in the pineal gland, as well as the hypothalamus (Table 2). The localization and functional data imply that SNORF36 is a non-rod, non-cone, ocular photoreceptor. It has been suggested that non-rod, non-cone ocular photoreceptors are responsible for photoentrainment and regulation of melatonin synthesis in the pineal gland (Freedman et al., 1999, Lucas et al., 1999).

Other regions containing high levels of SNORF36 RNA include trigeminal ganglia, spinal cord, and medulla. Localization to these structures as well as moderate levels in the dorsal root ganglia, strongly suggest a role in sensory transmission (or modulation) including nociceptive stimuli. Localization in the medulla also implies regulation of autonomic centers controlling respiration and cardiovascular function. Other CNS regions containing high levels of SNORF36 RNA include the amygdala, and substantia nigra. High levels in the amygdala and other limbic (or limbic related) structures suggests a role in modulation of mood, fear, phobia, anxiety and may provide a therapeutic target for the treatment of depression and other neuropsychiatric disorders. Localization to the substantia nigra (in conjunction with expression in the striatum) implies a role in regulation of dopaminergic systems, and may provide a therapeutic target for treatment of movement disorders such as Parkinsons disease or tardive dyskinesia.

-114-

5 The high levels of SNORF36 mRNA expressed in the hypothalamus described previously, in addition to the potential role in regulation of circadian rhythm, also indicates a role in neuroendocrine regulation, regulation of appetite and other functions that are modulated by the hypothalamus. High levels in the amygdala suggest a role in modulation of mood, fear, phobia, anxiety and may provide a therapeutic target for the treatment of depression and other neuropsychiatric disorders.

10 The presence of lower levels of SNORF36 RNA in other areas such as the hippocampal formation, olfactory bulb, cerebral cortex, cerebellum and other areas suggests multiple diverse functions as suggested in Table 2.

15 Non-neuronal tissue expressing high levels of SNORF36 RNA include the ovary and testes. Levels in these areas are comparable to many CNS regions including the cerebral cortex and cerebellum (Table 2). This strongly suggests a role in endocrine regulation or reproductive function, by neuronal or extraneuronal mechanisms. Other peripheral tissues showing moderate amounts of SNORF36 mRNA are listed in Table 2.

25 In summary, the high levels of SNORF36 RNA in the retina, pineal gland, and hypothalamus suggest a role as a non-rod, non-cone ocular photoreceptor involved in regulation of circadian rhythms. The distribution of rat SNORF36 mRNA throughout the CNS implies broad regulatory function in the nervous system. Potential functions include modulation of sensory transmission, modulation of extrapyramidal motor systems, and modulation of the limbic system. The ovary and testes are among multiple peripheral organs that may be regulated by SNORF36. Its presence, albeit at lower levels, 30 in other peripheral tissues implies a broad regulatory role in multiple organ systems.



5

Amounts of mRNA encoding human SNORF36 expressed as, mean (whole brain standard curve), % of highest expressing tissue (CPU + SEM).

15

-116-

Region	SNORF36 % of max	SNORF 36b % of max	SNORF 36a % of max	SNORF 36a % of SNORF36	Potential applica- tions
fetal kidney	0.61 $\pm 0.10$	not detecte d	0.61	100	Develop- mental disorders
fetal liver	0.04 $\pm 0.01$	0.01	0.03	63.08	Develop- mental disorders
fetal lung	0.42 $\pm 0.11$	0.24	0.18	42.79	Develop- mental disorders
heart	7.44 $\pm 2.46$	not detecte d	7.44	100	Cardio- vascular disorders
hippo- campus	26.28 $\pm 1.76$	10.52	15.76	59.96	Cognition/ memory
hypo- thalamus	0.77 $\pm 0.07$	0.04	0.72	94.49	appetite/ obesity, neuro- endocrine regulation
kidney	0.16 $\pm 0.04$	trace	0.16	100	Hyperten- sion, electro- lyte balance
liver	trace	trace	0.02	NA	Diabetes
lung	trace	trace	0.03	NA	Respira- tory disorders, asthma
pancreas	0.07 $\pm 0.01$	not detecte d	0.07	100	Diabetes, endocrine disorders

10

WO 00/66630

PCT/US00/12065

-118-

Region	SNORF36 % of max	SNORF 36b % of max	SNORF 36a % of max	SNORF 36a % of SNORF36	Potential applica- tions
thalamus	5.56 $\pm 0.70$	1.19	4.37	78.62	Sensory integra- tion disorders
whole brain	15.82 $\pm 2.28$	4.97	10.85	68.59	

**Table 2**

**Summary of distribution of mRNA coding for rat SNORF36 receptors**

5 mRNA encoding SNORF36 is expressed as % of highest expressing tissue: retina  $\pm$  SEM.

Tissue	SNORF36	Potential applications
adipose	1.93 $\pm 0.21$	metabolic disorders
adrenal cortex	2.62 $\pm 0.48$	regulation of steroid hormones
adrenal medulla	2.86 $\pm 0.24$	regulation of epinephrine release
amygdala	9.52 $\pm 0.75$	depression, phobias, anxiety, mood disorders
aorta	1.08 $\pm 0.07$	cardiovascular disorders
celiac plexus	1.58 $\pm 0.14$	modulation of autonomic function
cerebellum	4.81 $\pm 0.43$	motor coordination
cerebral cortex	6.35 $\pm 0.41$	Sensory and motor integration, cognition
choroid plexus	7.02 $\pm 0.93$	regulation of cerebrospinal fluid
colon	0.61 $\pm 0.05$	gastrointestinal disorders
dorsal root ganglia	4.18 $\pm 0.23$	sensory transmission
duodenum	0.86 $\pm 0.10$	gastrointestinal disorders
heart	1.79 $\pm 0.19$	cardiovascular indications
hippocampus	3.45 $\pm 0.37$	cognition/memory
hypothalamus	9.13 $\pm 0.62$	appetite/obesity, neuroendocrine regulation

Tissue	SNORF36	Potential applications
kidney	0.95	electrolyte balance,
	$\pm 0.07$	hypertension
liver	trace	diabetes
medulla	9.33	analgesia, motor
	$\pm 0.57$	coordination
nucleus accumbens	4.29	regulation of
	$\pm 0.36$	dopaminergic function, drug addiction, neuropsychiatric disorders
olfactory bulb	3.96	olfaction
	$\pm 0.33$	
ovary	6.83	reproductive function
	$\pm 0.66$	
pancreas	0.30	diabetes, endocrine
	$\pm 0.04$	disorders
pineal	8.37	regulation of melatonin
	$\pm 0.21$	release
pituitary	3.68	endocrine/neuroendocrine
	$\pm 0.37$	regulation
retina	100	visual disorders,
	$\pm 7.26$	circadian rhythms
salivary gland	1.26	
	$\pm 0.21$	
spinal cord	14.47	analgesia, sensory
	$\pm 0.44$	modulation and transmission
spleen	0.27	immune disorders
	$\pm 0.14$	
stomach	0.30	gastrointestinal
	$\pm 0.02$	disorders
striated muscle	2.08	musculoskeletal disorders
	$\pm 0.18$	
striatum	3.72	modulation of
	$\pm 0.67$	dopaminergic function, motor disorders

-121-

Tissue	SNORF36	Potential applications
substantia nigra	11.92 $\pm 2.46$	modulation of dopaminergic function, modulation of motor coordination
testes	5.48 $\pm 0.21$	reproductive function
thalamus	3.85 $\pm 0.16$	sensory integration disorders
thymus	3.07 $\pm 0.37$	immune disorders
trigeminal ganglia	19.13 $\pm 2.83$	sensory transmission
urinary bladder	1.48 $\pm 0.11$	urinary incontinence
uterus	1.16 $\pm 0.14$	reproductive disorders
vas deferens	2.85 $\pm 0.21$	reproductive function
whole brain	4.12 $\pm 0.39$	

#### Expression of SNORF36 mRNA in human retina

To determine if SNORF36 mRNA is expressed in retina, RT-PCR was performed in human retina cDNA using two pairs of SNORF36-specific primers. Single bands of the expected sizes were visualized following agarose gel electrophoresis for each primer pair. Somewhat weaker bands were observed in similar RT-PCR reactions using whole brain cDNA as the template. In contrast, no bands were observed in RT-PCR reactions in which water was used as the template. As both primer pairs were designed to cross introns, similar sized bands were not detected in RT-PCR reactions in which genomic DNA was used as the template. These results demonstrate that SNORF36 mRNA is expressed in human retina.





REFERENCES

Arnheiter, H., "Eyes viewed from the skin" *Nature* 391:632-633 (1998).

5 Blackshaw, S., and Snyder, S.H., "Parapinopsin, a novel catfish opsin localized to the parapineal organ, defines a new gene family" *J. Neurosci.* 17:8083-8092 (1997).

10 Blackshaw, S., and Snyder, S.H., "Encephalopsin: a novel mammalian extraretinal opsin discretely localized in the brain" *J. Neurosci.* 19: 3681-3690 (1999).

15 Bradford, M.M., "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding", *Anal. Biochem.* 72: 248-254 (1976).

20 Burns, C.C., et al., "Identification and deletion of sequences required for feline leukemia virus RNA packaging and construction of a high-titer feline leukemia virus packaging cell line", *Virology* 222(1): 14-20 (1996).

25 Bush, et al., "Nerve growth factor potentiates bradykinin-induced calcium influx and release in PC12 cells" *J. Neurochem.* 57: 562-574 (1991).

30 Campbell, et al., "Light treatment for sleep disorders: consensus report V. Age-related disturbances" *J. Biol. Rhythms* 10: 151-154 (1995).

Chan, T., et al., "Introduction of hydroxyl-bearing amino acids causes bathochromic spectral shifts in rhodopsin. Amino acid substitutions responsible for red-green color pigment spectral tuning" *J. Biol. Chem.* 267: 9478-9480 (1992).

-124-

Chu, Y.Y., et al., "Characterization of the rat A2a adenosine receptor gene", *DNA Cell Biol.* 15(4): 329-337 (1996).

5 Cohen, G.B., et al., "Constitutive activation of opsin: influence of charge at position 134 and size at position 296" *Biochem.* 32: 6111-6115 (1993).

10 Cooke, K.M., et al., "The effects of evening light exposure on the sleep of elderly women expressing sleep complaints" *J. Behav. Med.* 21: 103-114 (1998).

15 Dascal, N., et al., "Atrial G protein-activated K<sup>+</sup> channel: expression cloning and molecular properties" *Proc. Natl. Acad. Sci. USA* 90: 10235-10239 (1993).

Fong, T.M., et al., "Mutational analysis of neurokinin receptor function" *Can. J. Physiol. Pharmacol.* 73(7): 860-865 (1995).

20 Foster, R.G., "Shedding light on the biological clock" *Neuron* 20: 829-832 (1998).

25 Foster, R.G., et al., "Opsin localization and chromophore retinoids identified within the basal brain of the lizard *Anolis carolinensis*" *J. Comp. Physiol. A.* 172: 33-45 (1993).

30 Foster, R.G., et al., "Identification of vertebrate deep brain photoreceptors" *Neurosci. Biobehav. Rev.* 18: 541-546 (1994).

35 Franke, H., et al., "An improved low-permeability in vitro-model of the blood-brain barrier: transport studies on retinoids, sucrose, haloperidol, caffeine and mannitol" *Brain Res.* 818: 65-71 (1999).

-125-

- Freedman, M.S., et al., "Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors" *Science* 284: 502-504 (1999).
- 5 Gartner, W., and Towner, P., "Invertebrate visual pigments" *Photochem. Photobiol.* 62: 1-16 (1995).
- Grace, M.S., et al., "Light perception in the vertebrate brain: an ultrastructural analysis of opsin- and vasoactive  
10 intestinal peptide-immunoactive neurons in Iguanid lizards" *J. Comp. Neurol.* 367: 575-594 (1996).
- Graziano, M.P. et al., "The amino terminal domain of the glucagon-like peptide-1 receptor is a critical determinant  
15 of subtype specificity" *Receptors Channels* 4(1): 9-17 (1996).
- Guan, X.M., et al., "Determination of Structural Domains for G Protein Coupling and Ligand Binding in  $\beta 3$  - Adrenergic Receptor" *Mol. Pharmacol.* 48(3): 492-498 (1995).
- 20 Gundersen, C.B., et al., "Serotonin receptors induced by exogenous messenger RNA in *Xenopus* oocytes" *Proc. R. Soc. Lond. B. Biol. Sci.* 219(1214): 103-109 (1983).
- 25 Han, M., et al., "The effects of amino acid replacements of glycine 121 on transmembrane helix 3 of rhodopsin" *J. Biol. Chem.* 271: 32330-32226 (1996).
- 30 Hao, W., and Fong, H.K.W., "The endogenous chromophore of retinal G protein-coupled receptor opsin from the pigment epithelium" *J. Biol. Chem.* 274: 6085-6090 (1999).
- 35 Hara-Nishimura, I., et al., "Cloning and nucleotide sequence of cDNA for retinochrome, retinal photoisomerase from the squid retina" *FEBS Lett.* 271: 106-110 (1990).



5

10

15

20

Kochendoerfer, G.G., et al., "Hoe color visual pigments are tuned" *Trends Biol. Sci.* 24: 300-305 (1999).

25

30

35

-127-

Lack, L., and Wright, H., "The effect of evening bright light in delaying the circadian rhythms and lengthening the sleep of early morning awakening insomniacs" *Sleep* 16: 436-443 (1993).

5

Lazareno, S. and Birdsall, N.J.M. "Pharmacological characterization of acetylcholine stimulated [35S]-GTPγS binding mediated by human muscarinic m1-m4 receptors: antagonist studies", *Br. J. Pharmacology* 109: 1120-1127 (1993)

10

Lucas, R.J., et al., "Regulation of the mammalian pineal by non-rod, non-cone, ocular photoreceptors" *Science* 284: 505-507 (1999).

15

Max, M., et al., "Pineal opsin: a nonvisual opsin expressed in chick pineal" *Science* 267: 1502-1506 (1995).

20

Max, M., et al., "Light-dependent activation of rod transducin by pineal opsin" *J. Biol. Chem.* 273: 26820-26826 (1998).

25

Milligan, G., et al., Chimaeric G alpha proteins: their potential use in drug discovery" *Trends Pharmacol. Sci.* 20: 118-124 (1999).

30

Muller, P.J., and Wilson, B.C., "An update on the penetration depth of 630 nm light in normal and malignant human brain tissue *in vivo*" *Phys. Med. Biol.* 31: 1295-1297 (1986).

Murphy, P. J., and Campbell, S.S., "Enhanced performance in elderly subjects following bright light treatment of sleep maintenance insomnia" *J. Sleep Res.* 5: 165-172 (1996).

35

Nobes, C., et al., "Activation of the GTP-binding protein Gq

-128-

by rhodopsin in squid photoreceptors" *Biochem. J.* 287: 545-548 (1992).

5 Okano, T., et al., "Pinopsin is a chicken pineal photoreceptive molecule" *Nature* 372: 94-97 (1994).

Oren, D.A., et al., "Treatment of seasonal affective disorder with green light and red light", *Am. J. Psychiatry* 148: 509-511 (1991).

10

Pardridge, W.M., et al., "Restricted transport of vitamin D and A derivatives through the rat blood-brain barrier" *J. Neurochem.* 44: 1138-1141 (1985).

15

Partonen, T., "Short note: magnetoreception attributed to the efficacy of light therapy" *Med. Hypotheses* 51: 447-448 (1998).

20

Provencio, I., et al., "Melanopsin: an opsin in melanophores, brain and eye" *Proc. Natl. Acad. Sci. USA* 95: 340-345 (1998).

25

Quick, M.W. and Lester, H.A., "Methods for expression of excitability proteins in *Xenopus* oocytes", *Meth. Neurosci.* 19: 261-279 (1994).

Refinetti, R., "Chronobiology: Business and Health Care" *Circadian Physiology*: pp. 127-160, CRC Press (1999).

30

Rollag, M.D., "Amphibian melanophores become photosensitive when treated with retinal" *J. Exp. Zoology* 275: 20-26 (1996).

Rosenthal, N.E., et al., "Phase-shifting effects of bright morning light as treatment for delayed sleep phase syndrome" *Sleep* 13: 354-361 (1990).

35

-129-

Salon, J.A. and Owicki, J.A., "Real-time measurements of receptor activity: Application of microphysiometric techniques to receptor biology" *Methods in Neuroscience* 25: 201-224, Academic Press (1996).

5

Sanger, F., et al., "DNA sequencing with chain-terminating inhibitors" *Proc. Natl. Acad. Sci. USA* 74: 5463-5467 (1977).

10

Sedgwick, P.M., "Disorders of the sleep-wake cycle in adults" *Postgrad. Med. J.* 74: 134-138 (1998).

15

Silver, R., et al., "Coexpression of opsin- and VIP-like-immunoreactivity in CSF-contacting neurons of the avian brain" *Cell Tiss. Res.* 253: 189-198 (1988).

20

Smith, K.E., et al., "Expression cloning of a rat hypothalamic galanin receptor coupled to phosphoinositide turnover." *J. Biol. Chem.* 272: 24612-24616 (1997).

Soni, B.G., et al., "Novel retinal photoreceptors" *Nature* 394: 27-28 (1998).

25

Soni, B.G., et al., "A novel and ancient vertebrate opsin" *FEBS Lett.* 407: 279-283 (1997).

30

Spurney, R.F., et al., "The C-terminus of the thromboxane receptor contributes to coupling and desensitization in a mouse mesangial cell line", *J. Pharmacol. Exp. Ther.* 283(1): 207-215 (1997).

35

-130-

Surya, A., and Knox, B.E., "Enhancement of opsin activity by all-trans-retinal" *Exp. Eye Res.* 66: 599-603 (1998).

5 Surya, A., et al., "Transducin activation by the bovine opsin apoprotein" *J. Biol. Chem.* 270: 5024-5031 (1995).

10 Takahashi, T., et al., "Rat brain serotonin receptors in *Xenopus* oocytes are coupled by intracellular calcium to endogenous channels." *Proc. Natl. Acad. Sci. USA* 84(14): 5063-5067 (1987).

15 Takanaka, Y., et al., "Light-dependent expression of pinopsin gene in chicken pineal gland" *J. Neurochem.* 70: 908-913 (1998).

20 Tanaka, T., et al., "Lipocalin-type prostaglandin D-synthase (beta-trace) is a newly recognized type of retinoid transporter" *J. Biol. Chem.* 272: 15789-15795 (1997).

25 Tao, L., et al., "Structure and developmental expression of the mouse RGR opsin gene" *Mol. Vision* 4: 25-30 (1998).

30 Terakita, A., et al., "Selective activation of G-protein subtypes by vertebrate and invertebrate rhodopsins" *FEBS Lett.* 439: 110-114 (1998).

35 Terman, M., and Terman, J.S., "Bright light therapy: side effects and benefits across the symptom spectrum" *J. Clin. Psychiatry* 60: 799-808 (1999).

40 Tian, W., et al., "Determinants of alpha-Adrenergic Receptor Activation of G protein: Evidence for a Precoupled Receptor/G protein State." *Molecular Pharmacology* 45: 524-553 (1994).

45 Underwood, D.J. et al., "Structural model of antagonist and



-131-

agonist binding to the angiotensin II, AT1 subtype, G protein coupled receptor", *Chem. Biol.* 1(4): 211-221 (1994).

5 Wade, P.D., et al., "Mammalian cerebral cortical tissue responds to low-intensity visible light" *Proc. Natl. Acad. Sci. USA* 85: 9322-9326 (1988).

10 Wood, S.F., et al., "Inositol triphosphate production in squid photoreceptors. Activation by light, aluminium fluoride, and guanine nucleotides" *J. Biol. Chem.* 264: 12970-12976 (1989).

15 Yarfitz, S., and Hurley, J.B., "Transduction mechanisms of vertebrate and invertebrate photoreceptors" *J. Biol. Chem.* 269: 14329-14332 (1994).

20 Yoshikawa, T., and Oishi, T., "Extraretinal photoreception and circadian systems in nonmammalian vertebrates" *Comp. Biochem. Physiol.* 119B: 65-72 (1998).

-132-

What is claimed is:

1. An isolated nucleic acid encoding a mammalian SNORF36 receptor.
2. The nucleic acid of claim 1, wherein the nucleic acid is DNA.
3. The DNA of claim 2, wherein the DNA is cDNA.
4. The DNA of claim 2, wherein the DNA is genomic DNA.
5. The nucleic acid of claim 1, wherein the nucleic acid is RNA.
6. The nucleic acid of claim 1, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor.
7. The nucleic acid of claim 1, wherein the mammalian SNORF36 receptor is a human SNORF36b receptor.
8. The nucleic acid of claim 6, wherein the human SNORF36a receptor has an amino acid sequence identical to that encoded by the plasmid pcDNA3.1-hSNORF36a-f (ATCC Accession No. PTA-203977).
9. The nucleic acid of claim 7, wherein the human SNORF36b receptor has an amino acid sequence identical to that encoded by the plasmid pcDNA3.1-hSNORF36b-f (ATCC Accession No. PTA-203976).
10. The nucleic acid of claim 1, wherein the mammalian SNORF36 receptor is a rat SNORF36 receptor.
11. The nucleic acid of claim 10, wherein the rat SNORF36 receptor has an amino acid sequence identical to that

-133-

encoded by the plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

- 5 12. The nucleic acid of claim 6, wherein the human SNORF36a receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 2A-2B (SEQ ID NO: 2).
- 10 13. The nucleic acid of claim 7, wherein the human SNORF36b receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 4A-4C (SEQ ID NO: 4).
- 15 14. The nucleic acid of claim 10, wherein the rat SNORF36 receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 10A-10C (SEQ ID NO: 8).
- 20 15. A purified mammalian SNORF36 receptor protein.
16. The purified mammalian SNORF36 receptor protein of claim 15, wherein the SNORF36 receptor protein is a human SNORF36a receptor protein.
- 25 17. The purified mammalian SNORF36 receptor protein of claim 15, wherein the SNORF36 receptor protein is a human SNORF36b receptor protein.
- 30 18. The purified mammalian SNORF36 receptor protein of claim 15, wherein the SNORF36 receptor protein is a rat SNORF36 receptor protein.
19. A vector comprising the nucleic acid of claim 1.
- 35 20. A vector comprising the nucleic acid of claim 6 or claim 7.

-134-

21. A vector comprising the nucleic acid of claim 10.
22. A vector of claim 19, 20 or 21 adapted for expression  
in a cell which comprises the regulatory elements  
necessary for expression of the nucleic acid in the  
cell operatively linked to the nucleic acid encoding  
the receptor so as to permit expression thereof,  
wherein the cell is a bacterial, amphibian, yeast,  
insect or mammalian cell.
23. The vector of claim 22, wherein the vector is a  
baculovirus.
24. The vector of claim 19, wherein the vector is a  
plasmid.
25. The plasmid of claim 24 designated pCDNA3.1-hSNORF36a-f  
(ATCC Accession No. PTA-203977)
26. The plasmid of claim 24 designated pCDNA3.1-hSNORF36b-f  
(ATCC Accession No. PTA-203976).
27. The plasmid of claim 24 designated pEXJ.T7-rSNORF36-f  
(ATCC Patent Depository No. PTA-1216).
28. A cell comprising the vector of claim 22.
29. A cell of claim 28, wherein the cell is a non-mammalian  
cell.
30. A cell of claim 29, wherein the non-mammalian cell is  
a *Xenopus* oocyte cell or a *Xenopus* melanophore cell.
31. A cell of claim 28, wherein the cell is a mammalian  
cell.

-135-

32. A mammalian cell of claim 31, wherein the cell is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell, a LM(tk-) cell, a mouse Y1 cell, or a CHO cell.
- 5 33. A cell of claim 28, wherein the cell is an insect cell.
34. An insect cell of claim 33, wherein the insect cell is an Sf9 cell, an Sf21 cell or a Trichoplusia ni 5B-4 cell.
- 10 35. A membrane preparation isolated from the cell of any one of claims 28, 29, 31, 32, 33 or 34.
- 15 36. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within one of the two strands of the nucleic acid encoding the human SNORF36a receptor contained in plasmid pCDNA3.1-hSNORF36a-f (ATCC Accession No. PTA-203977).
- 20 37. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a unique sequence present within one of the two strands of the nucleic acid encoding the human SNORF36b receptor contained in plasmid pCDNA3.1-hSNORF36b-f (ATCC Accession No. PTA-203976).
- 25 38. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a
- 30
- 35

-136-

unique sequence present within one of the two strands of the nucleic acid encoding the mammalian SNORF36 receptor contained in plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).

5

39. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a  
10 unique sequence present within (a) the nucleic acid sequence shown in Figures 1A-1C (SEQ ID NO: 1) or (b) the reverse complement thereof.

15

40. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a  
20 unique sequence present within (a) the nucleic acid sequence shown in Figures 3A-3C (SEQ ID NO: 3) or (b) the reverse complement thereof.

25

41. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF36 receptor, wherein the probe has a sequence complementary to a  
25 unique sequence present within (a) the nucleic acid sequence shown in Figures 9A-9C (SEQ ID NO: 7) or (b) the reverse complement thereof.

30

42. The nucleic acid probe of claim 39, 40 or 41, wherein the nucleic acid is DNA.

35

43. The nucleic acid probe of claim 39, 40 or 41, wherein the nucleic acid is RNA.

44. An antisense oligonucleotide having a sequence capable

-137-

of specifically hybridizing to the RNA of claim 5, so as to prevent translation of the RNA.

- 5 45. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA of claim 4, so as to prevent transcription of the genomic DNA.
- 10 46. An antisense oligonucleotide of claim 44 or 45, wherein the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.
- 15 47. An antibody capable of binding to a mammalian SNORF36 receptor encoded by the nucleic acid of claim 1.
- 20 48. An antibody of claim 47, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.
- 25 49. An agent capable of competitively inhibiting the binding of the antibody of claim 47 to a mammalian SNORF36 receptor.
- 30 50. An antibody of claim 47, wherein the antibody is a monoclonal antibody or antisera.
- 35 51. A pharmaceutical composition comprising (a) an amount of the oligonucleotide of claim 44 capable of passing through a cell membrane and effective to reduce expression of a mammalian SNORF36 receptor and (b) a pharmaceutically acceptable carrier capable of passing through the cell membrane.
52. A pharmaceutical composition of claim 51, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

-138-

53. A pharmaceutical composition of claim 52, wherein the substance which inactivates mRNA is a ribozyme.

5 54. A pharmaceutical composition of claim 52, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a mammalian SNORF36 receptor on a cell capable of being taken up by the cells after binding to the structure.

10 55. A pharmaceutical composition of claim 54, wherein the pharmaceutically acceptable carrier is capable of binding to a mammalian SNORF36 receptor which is specific for a selected cell type.

15 56. A pharmaceutical composition which comprises an amount of the antibody of claim 47 effective to block binding of a ligand to a human SNORF36 receptor and a pharmaceutically acceptable carrier.

20 57. A transgenic, nonhuman mammal expressing DNA encoding a mammalian SNORF36 receptor of claim 1.

25 58. A transgenic, nonhuman mammal comprising a homologous recombination knockout of the native mammalian SNORF36 receptor.

30 59. A transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to the DNA encoding a mammalian SNORF36 receptor of claim 1 so placed within the genome as to be transcribed into antisense mRNA which is complementary to and hybridizes with mRNA encoding the mammalian SNORF36 receptor so as to thereby reduce translation of such mRNA and expression of such receptor.

35 60. The transgenic, nonhuman mammal of claim 57 or 58,



-139-

wherein the DNA encoding the mammalian SNORF36 receptor additionally comprises an inducible promoter.

- 5 61. The transgenic, nonhuman mammal of claim 57 or 58, wherein the DNA encoding the mammalian SNORF36 receptor additionally comprises tissue specific regulatory elements.
- 10 62. A transgenic, nonhuman mammal of claim 57, 58, or 59, wherein the transgenic, nonhuman mammal is a mouse.
- 15 63. A process for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises contacting cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to  
20 the mammalian SNORF36 receptor.
- 25 64. A process for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises contacting a membrane preparation from cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with the compound under conditions suitable for binding, and detecting specific binding of  
30 the chemical compound to the mammalian SNORF36 receptor.
- 35 65. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor.

-140-

- 5 66. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has substantially the same amino acid sequence as the human SNORF36a receptor encoded by plasmid pCDNA3.1-hSNORF36a-f (ATCC Accession No. PTA-203977).
- 10 67. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has substantially the same amino acid sequence as the human SNORF36b receptor encoded by the plasmid pCDNA3.1-hSNORF36b-f (ATCC Accession No. PTA-203976).
- 15 68. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has substantially the same amino acid sequence as that shown in Figures 2A-2B (SEQ ID NO: 2) or Figures 4A-4C (SEQ ID NO: 4).
- 20 69. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has the amino acid sequence shown in Figures 2A-2B (SEQ ID NO: 2) or Figures 4A-4C (SEQ ID NO: 4).
- 25 70. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor is a rat SNORF36 receptor.
- 30 71. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has substantially the same amino acid sequence as the rat SNORF36 receptor encoded by plasmid pEXJ.T7-rSNORF36-f (ATCC Patent Depository No. PTA-1216).
- 35 72. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has substantially the same amino acid sequence as that shown in Figures 10A-10C (SEQ ID NO: 8).

-141-

73. The process of claim 63 or 64, wherein the mammalian SNORF36 receptor has the same amino acid sequence as that shown in Figures 10A-10C (SEQ ID NO: 8).
- 5 74. The process of claim 63 or 64, wherein the compound is not previously known to bind to a mammalian SNORF36 receptor.
75. A compound identified by the process of claim 74.
- 10 76. A process of claim 63 or 64, wherein the cell is an insect cell.
77. The process of claim 63 or 64, wherein the cell is a mammalian cell.
- 15 78. The process of claim 77, wherein the cell is nonneuronal in origin.
- 20 79. The process of claim 78, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.
- 25 80. A process of claim 77, wherein the compound is a compound not previously known to bind to a mammalian SNORF36 receptor.
81. A compound identified by the process of claim 80.
- 30 82. A process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises separately contacting cells expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not
- 35 normally express the mammalian SNORF36 receptor, with

-142-

both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF36 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF36 receptor.

83. A process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF36 receptor which comprises separately contacting a membrane preparation from cells expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF36 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF36 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF36 receptor.

84. A process of claim 82 or 83, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor.

85. A process of claim 82 or 83, wherein the mammalian SNORF36 receptor is a rat SNORF36 receptor.

-143-

86. The process of claim 82 or 83, wherein the cell is an insect cell.
87. The process of claim 82 or 83, wherein the cell is a mammalian cell.
88. The process of claim 87, wherein the cell is nonneuronal in origin.
89. The process of claim 88, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.
90. The process of claim 89, wherein the compound is not previously known to bind to a mammalian SNORF36 receptor.
91. A compound identified by the process of claim 90.
92. A method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF36 receptor to identify a compound which specifically binds to the mammalian SNORF36 receptor, which comprises
- (a) contacting cells transfected with, and expressing, DNA encoding the mammalian SNORF36 receptor with a compound known to bind specifically to the mammalian SNORF36 receptor;
  - (b) contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian SNORF36 receptor, under conditions permitting binding of compounds known to bind to the mammalian SNORF36 receptor;

-144-

- (c) determining whether the binding of the compound known to bind to the mammalian SNORF36 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so
- (d) separately determining the binding to the mammalian SNORF36 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF36 receptor.
93. A method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF36 receptor to identify a compound which specifically binds to the mammalian SNORF36 receptor, which comprises
- (a) contacting a membrane preparation from cells transfected with, and expressing, DNA encoding the mammalian SNORF36 receptor with the plurality of compounds not known to bind specifically to the mammalian SNORF36 receptor under conditions permitting binding of compounds known to bind to the mammalian SNORF36 receptor;
- (b) determining whether the binding of a compound known to bind to the mammalian SNORF36 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so
- (c) separately determining the binding to the mammalian SNORF36 receptor of each compound

-145-

included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF36 receptor.

5

94. A method of claim 92 or 93, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor or a human SNORF36b receptor.

10

95. A method of claim 92 or 93, wherein the mammalian SNORF36 receptor is a rat SNORF36 receptor.

96. A method of claim 92 or 93, wherein the cell is a mammalian cell.

15

97. A method of claim 96, wherein the mammalian cell is non-neuronal in origin.

98. The method of claim 97, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell, or an NIH-3T3 cell.

20

99. A method of detecting expression of a mammalian SNORF36 receptor by detecting the presence of mRNA coding for the mammalian SNORF36 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of claim 36, 37, 38, 39, 40 or 41 under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the mammalian SNORF36 receptor by the cell.

25

30

100. A method of detecting the presence of a mammalian SNORF36 receptor on the surface of a cell which comprises contacting the cell with the antibody of

35

-146-

claim 47 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of the mammalian SNORF36 receptor on the surface of the cell.

- 5
101. A method of determining the physiological effects of varying levels of activity of mammalian SNORF36 receptors which comprises producing a transgenic, nonhuman mammal of claim 57 whose levels of mammalian SNORF36 receptor activity are varied by use of an inducible promoter which regulates mammalian SNORF36 receptor expression.
- 10
102. A method of determining the physiological effects of varying levels of activity of mammalian SNORF36 receptors which comprises producing a panel of transgenic, nonhuman mammals of claim 57 each expressing a different amount of mammalian SNORF36 receptor.
- 15
- 20
103. A method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor comprising administering a compound to the transgenic, nonhuman mammal of claim 57, 58, or 59, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal as a result of overactivity of a mammalian SNORF36 receptor, the alleviation of such an abnormality identifying the compound as an antagonist.
- 25
- 30
104. The method of claim 103, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.
- 35



-147-

105. An antagonist identified by the method of claim 103.

106. A composition comprising an antagonist of claim 105 and a carrier.

5

107. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition of claim 106, thereby treating the abnormality.

10

108. A method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor comprising administering a compound to the transgenic, nonhuman mammal of claim 57, 58, or 59, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal, the alleviation of such an abnormality identifying the compound as an agonist.

15

20

109. The method of claim 108, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

25

110. An agonist identified by the method of claim 109.

111. A composition comprising an agonist identified by the method of claim 110 and a carrier.

30

112. A method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject an effective

35

-148-

amount of the composition of claim 111 so as to thereby treat the abnormality.

113. A method for diagnosing a predisposition to a disorder associated with the activity of a specific mammalian allele which comprises:

- (a) obtaining DNA of subjects suffering from the disorder;
- (b) performing a restriction digest of the DNA with a panel of restriction enzymes;
- (c) electrophoretically separating the resulting DNA fragments on a sizing gel;
- (d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian SNORF36 receptor and labeled with a detectable marker;
- (e) detecting labeled bands which have hybridized to the DNA encoding a mammalian SNORF36 receptor of claim 1 to create a unique band pattern specific to the DNA of subjects suffering from the disorder;
- (f) repeating steps (a)-(e) with DNA obtained for diagnosis from subjects not yet suffering from the disorder; and
- (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) with the band pattern from step (f) for subjects not yet suffering from the disorder so as

-149-

to determine whether the patterns are the same or different and thereby diagnose predisposition to the disorder if the patterns are the same.

- 5        114. The method of claim 113, wherein a disorder associated with the activity of a specific mammalian allele is diagnosed.
- 10       115. A method of preparing the purified mammalian SNORF36 receptor of claim 15 which comprises:
- (a) culturing cells which express the mammalian SNORF36 receptor;
  - 15       (b) recovering the mammalian SNORF36 receptor from the cells; and
  - (c) purifying the mammalian SNORF36 receptor so recovered.
- 20       116. A method of preparing the purified mammalian SNORF36 receptor of claim 15 which comprises:
- 25       (a) inserting a nucleic acid encoding the mammalian SNORF36 receptor into a suitable expression vector;
  - (b) introducing the resulting vector into a suitable host cell;
  - 30       (c) placing the resulting host cell in suitable conditions permitting the production of the mammalian SNORF36 receptor;
  - 35       (d) recovering the mammalian SNORF36 receptor so produced; and optionally

-150-

(e) isolating and/or purifying the mammalian SNORF36 receptor so recovered.

5 117. A process for determining whether a chemical compound is a mammalian SNORF36 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF36 receptor with the compound under conditions permitting the activation of the mammalian SNORF36 receptor, and detecting any  
10 increase in mammalian SNORF36 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF36 receptor agonist.

15 118. A process for determining whether a chemical compound is a mammalian SNORF36 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF36 receptor with the compound in the presence of a known mammalian SNORF36 receptor agonist, under conditions permitting  
20 the activation of the mammalian SNORF36 receptor, and detecting any decrease in mammalian SNORF36 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF36 receptor antagonist.

25 119. A process of claim 117 or 118, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

30 120. A composition which comprises an amount of a mammalian SNORF36 receptor agonist determined by the process of claim 117 effective to increase activity of a mammalian SNORF36 receptor and a carrier.

35 121. A composition of claim 120, wherein the mammalian SNORF36 receptor agonist is not previously known.

-151-

122. A composition which comprises an amount of a mammalian SNORF36 receptor antagonist determined by the process of claim 118 effective to reduce activity of a mammalian SNORF36 receptor and a carrier.
- 5 123. A composition of claim 122, wherein the mammalian SNORF36 receptor antagonist is not previously known.
- 10 124. A process for determining whether a chemical compound specifically binds to and activates a mammalian SNORF36 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36
- 15 receptor, with the chemical compound under conditions suitable for activation of the mammalian SNORF36 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in
- 20 the presence of the chemical compound indicating that the compound activates the mammalian SNORF36 receptor.
125. The process of claim 124, wherein the second messenger response comprises chloride channel activation and the
- 25 change in second messenger is an increase in the level of chloride current.
126. The process of claim 124, wherein the second messenger response comprises intracellular calcium levels and the
- 30 change in second messenger is an increase in the measure of intracellular calcium.
127. The process of claim 124, wherein the second messenger response comprises release of inositol phosphate and
- 35 the change in second messenger is an increase in the level of inositol phosphate.

-152-

128. A process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian SNORF36 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF36 receptor, wherein such cells do not normally express the mammalian SNORF36 receptor, with both the chemical compound and a second chemical compound known to activate the mammalian SNORF36 receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian SNORF36 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian SNORF36 receptor.
129. The process of claim 128, wherein the second messenger response comprises chloride channel activation and the change in second messenger response is a smaller increase in the level of chloride current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.
130. The process of claim 129, wherein the second messenger response comprises change in intracellular calcium levels and the change in second messenger response is a smaller increase in the measure of intracellular calcium in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

-153-

131. The process of claim 128, wherein the second messenger response comprises release of inositol phosphate and the change in second messenger response is a smaller increase in the level of inositol phosphate in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.
132. A process of any of claims 124, 125, 126, 127, 128, 129, 130, or 131, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.
133. The process of any one of claims 121, 125, 126, 127, 128, 129, 130, or 131, wherein the cell is an insect cell.
134. The process of any one of claims 124, 125, 126, 127, 128, 129, 130, or 131, wherein the cell is a mammalian cell.
135. The process of claim 134, wherein the mammalian cell is nonneuronal in origin.
136. The process of claim 135, wherein the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.
137. The process of claim 124, 125, 126, 127, 128, 129, 130, or 131, wherein the compound is not previously known to bind to a mammalian SNORF36 receptor.
138. A compound determined by the process of claim 137.
139. A composition which comprises an amount of a mammalian SNORF36 receptor agonist determined to be such by the

-154-

process of claim 124, 125, 126, or 127, effective to increase activity of the mammalian SNORF36 receptor and a carrier.

5       140. A composition of claim 139, wherein the mammalian SNORF36 receptor agonist is not previously known.

10       141. A composition which comprises an amount of a mammalian SNORF36 receptor antagonist determined to be such by the process of claim 128, 129, 130, or 131, effective to reduce activity of the mammalian SNORF36 receptor and a carrier.

15       142. A composition of claim 141, wherein the mammalian SNORF36 receptor antagonist is not previously known.

20       143. A method of screening a plurality of chemical compounds not known to activate a mammalian SNORF36 receptor to identify a compound which activates the mammalian SNORF36 receptor which comprises:

25           (a) contacting cells transfected with and expressing the mammalian SNORF36 receptor with the plurality of compounds not known to activate the mammalian SNORF36 receptor, under conditions permitting activation of the mammalian SNORF36 receptor;

30           (b) determining whether the activity of the mammalian SNORF36 receptor is increased in the presence of one or more of the compounds; and if so

35           (c) separately determining whether the activation of the mammalian SNORF36 receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian SNORF36 receptor.



-155-

144. A method of claim 143, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

5 145. A method of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian SNORF36 receptor to identify a compound which inhibits the activation of the mammalian SNORF36 receptor, which comprises:

10

(a) contacting cells transfected with and expressing the mammalian SNORF36 receptor with the plurality of compounds in the presence of a known mammalian SNORF36 receptor agonist, under conditions  
15 permitting activation of the mammalian SNORF36 receptor;

20

(b) determining whether the extent or amount of activation of the mammalian SNORF36 receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian SNORF36 receptor in the absence of such one or more compounds; and if  
25 so

30

(c) separately determining whether each such compound inhibits activation of the mammalian SNORF36 receptor for each compound included in the plurality of compounds, so as to thereby identify  
35 any compound included in such plurality of compounds which inhibits the activation of the mammalian SNORF36 receptor.

40

146. A method of claim 145, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or rat SNORF36 receptor.

-156-

147. A method of any one of claims 143, 144, 145 or 146, wherein the cell is a mammalian cell.

5 148. A method of claim 147, wherein the mammalian cell is non-neuronal in origin.

149. The method of claim 148, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

10 150. A composition comprising a compound identified by the method of claim 143 or 144 in an amount effective to increase mammalian SNORF36 receptor activity and a carrier.

15 151. A composition comprising a compound identified by the method of claim 145 or 146 in an amount effective to decrease mammalian SNORF36 receptor activity and a carrier.

20 152. A method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject a compound which is a mammalian SNORF36 receptor agonist in an amount effective to treat the abnormality.

25 153. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF36 receptor which comprises administering to the subject a compound which is a mammalian SNORF36 receptor antagonist in an amount effective to treat the abnormality.

30 154. A process for making a composition of matter which specifically binds to a mammalian SNORF36 receptor

-157-

which comprises identifying a chemical compound using the process of any one of claims 63, 64, 82, 83, 92 or 93 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

155. The process of claims 154, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

156. A process for making a composition of matter which specifically binds to a mammalian SNORF36 receptor which comprises identifying a chemical compound using the process of any of claims 117, 124, or 143 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

157. The process of claim 156, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

158. A process for making a composition of matter which specifically binds to a mammalian SNORF36 receptor which comprises identifying a chemical compound using the process of any of claims 118, 128 or 145 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

159. The process of claim 158, wherein the mammalian SNORF36 receptor is a human SNORF36a receptor, a human SNORF36b receptor or a rat SNORF36 receptor.

160. A process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by the process of any of claims 63, 64, 82, 83, 92 or 93 or a novel

-158-

structural and functional analog or homolog thereof.

161. The process of claim 160, wherein the mammalian SNORF36  
receptor is a human SNORF36a receptor, a human  
5 SNORF36b receptor or a rat SNORF36 receptor.

162. A process for preparing a composition which comprises  
admixing a carrier and a pharmaceutically effective  
amount of a chemical compound identified by the process  
10 of any of claims 117, 124, or 143 or a novel structural  
and functional analog or homolog thereof.

163. The process of claim 162, wherein the mammalian SNORF36  
receptor is a human SNORF36a receptor, a human SNORF36b  
15 receptor or a rat SNORF36 receptor.

164. A process for preparing a composition which comprises  
admixing a carrier and a pharmaceutically effective  
amount of a chemical compound identified by the process  
20 of any of claims 118, 128 or 145 or a novel structural  
and functional analog or homolog thereof.

165. The process of claim 164, wherein the mammalian SNORF36  
receptor is a human SNORF36a receptor, a human  
25 SNORF36b receptor or a rat SNORF36 receptor.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>C07K 14/705, C07H 21/04, C12N 15/63, 1/21, C12P 21/02, G01N 33/53</b>		<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/66630</b> <b>(43) International Publication Date:</b> 9 November 2000 (09.11.00)
<b>(21) International Application Number:</b> PCT/US00/12065 <b>(22) International Filing Date:</b> 3 May 2000 (03.05.00) <b>(30) Priority Data:</b> 09/303,593 3 May 1999 (03.05.99) US 09/518,914 3 March 2000 (03.03.00) US <b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications</b> US 09/303,593 (CIP) Filed on 3 May 1999 (03.05.99) US 09/518,914 (CIP) Filed on 3 March 2000 (03.03.00) <b>(71) Applicant (for all designated States except US):</b> SYNAPTIC PHARMACEUTICAL CORPORATION [US/US]; 215 College Road, Paramus, NJ 07652 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> BOROWSKY, Beth, E. [US/US]; 218 Park Street, Montclair, NJ 07042 (US). OGOZALEK, Kristine, L. [US/US]; 25 Durand Place, Rochelle Park, NJ 07662 (US). LAKHLANI, Parul, P. [IN/US]; 304 Spring Valley Road, Paramus, NJ 07652 (US).			<b>ADHAM, Nika</b> [US/US]; 301 Mastin Place, Ridgewood, NJ 07450 (US). <b>(74) Agent:</b> WHITE, John, P.; Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, NY 10036 (US). <b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the 'expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> DNA ENCODING SNORF36a AND SNORF36b RECEPTORS			
<b>(57) Abstract</b>  This invention provides isolated nucleic acids encoding mammalian SNORF36 receptors, purified mammalian SNORF36 receptors, vectors comprising nucleic acid encoding mammalian SNORF36 receptors, cells comprising such vectors, antibodies directed to mammalian SNORF36 receptors, nucleic acid probes useful for detecting nucleic acid encoding mammalian SNORF36 receptors, antisense oligonucleotides complementary to unique sequences of nucleic acid encoding mammalian SNORF36 receptors, transgenic, nonhuman animals which express DNA encoding normal or mutant mammalian SNORF36 receptors, methods of isolating mammalian SNORF36 receptors, methods of treating an abnormality that is linked to the activity of the mammalian SNORF36 receptors, as well as methods of determining binding of compounds to mammalian SNORF36 receptors, methods of identifying agonists and antagonists of SNORF36 receptors, and agonists and antagonists so identified.			

10/018192

## FIGURE 1A

1 CAACTCAGGATGAACCTCCTTCGGGGCCAAGAGTCCCGCCAGCCCAACCAAGAGCCC 60  
61 AGCTGCATGGCCACCCAGCACCCAGCTGGTGGACAGCTCCAGAGCAGCATCTCC 120  
121 AGCCTGGGCGGCTTCCATCCATCAGTCCACAGCACCTGGGACTTGGGCTGCTGCCTGG 180  
181 GTCCCCCTCCCCACGGTTGA.GTTCAGACCATGCCCACTATACCCCTGGGCACAGTGATC 240  
241 TTGCTGGTGGGACTACGGGGATGCTGGCAACCTGACGGTCATCTATACCTTCTGCAGG 300  
301 AGCAGAAGCCCTCCGGACACCTGCCCAACATGTTCAATTATCAACCTCGCGGTCAGCGACTTC 360  
361 CTCATGTCCTTACCCAGGCCCTGTCTTCTTCACCAGTAGCCCTCTATAAGCAGTGGCTC 420  
421 TTTGGGAGACAGGCTGCGAGTTCTATGCCCTTCTGTGGAGCTCTCTTTGGCATTTCCCTCC 480  
481 ATGATCACCCCTGACGGCCATCGCCCTGGACCGCTACCTGGTAATCACACGCCCGCTGGCC 540  
541 ACCTTTGGTGTGGCGTCCAAGAGGCGTGCGGCATTGTCTCCTGCTGGCGGTTTGGCTCTAT 600  
601 GCCCTGGCCTGGAGTCTGCCACCCCTTCTTCGGCTGGAGCGCCCTACGTGCCCGGAGGGGTG 660  
661 CTGACATCCTGCTCCTGGGACTACATGAGCTTCACGCCCGCGCGTGGTGCTACACCATG 720

1/35

2/35

721	CTTCTCTGCTGCTTCGTGTCTTCCCTCCCTCTGCTTATCATCATCTACTGTACTCATCTTC	780
781	ATCTTCAGGGCCATCCGGGAGACAGGAGCGGCTCTCCAGACCTTCGGGGCCTGCAAGGC	840
841	AATGGCCGAGTCCCCTGTGGCAGCGGCAGCGGCTGCAGAGCGAGTGCAAGATGGCCAAGATC	900
901	ATGCTGCTGGTCACTCCTCTTTCGTGCTCTCTGGGCTCCCTATTCCGCTGTGGCCCCTG	960
961	GTGGCCCTTGTGCTGGGTACGCACACGTCCTGACACCCCTACATGAGCTCGGTGCCAGCCGTC	1020
1021	ATCGCCAAGGCCCTCTGCAATCCACAACCCCATCATTTACGCCATCACCCACCCCAAGTAC	1080
1081	AGGGTGGCCATTGCCCCAGCACCTGCCCTGCCCTGGGGTGTCTGGGTGTATCACGCCCGG	1140
1141	CACAGTCGCCCCCTACCCCCAGCTACCGCTCCACCCACCGCTCCACGCTGACCAGCCACACC	1200
1201	TCCAACCTCAGCTGGATCTCCATACGGAGGCGCCAGGAGTCCCTGGGCTCGGAGAGTGAG	1260
1261	GTGGGCTGGACACACATGGAGGCAGCAGCTGTGTGGGGAGCTGCCAGCAAGCAATGGG	1320
1321	CGGTCCCCTCTACGGTCAAGGTCTGGAGGACTTGGAAGCCAAGCACCCCCCAGACCCCAG	1380
1381	GGACACGAAGCAGAGACTCCAGGGAAGACCAAGGGGTGATCCCCAGCCAGGACCCCAGG	1440

10/018192

3/35

FIGURE 1C

1441 ATGTAGGACGCCCACTGGCTCTCCCTTTCTTCTGAGACACATCCAGCCCCCCCCACGTCTC 1500  
1501 CCTCATAT 1508



FIGURE 2A

4/35

1	M	N	P	P	S	G	P	R	V	P	P	S	P	T	Q	E	P	S	C	M	20
21	A	T	P	A	P	S	W	W	D	S	S	Q	S	S	I	S	S	L	G	40	
41	R	L	P	S	I	S	P	T	A	P	G	T	W	A	A	A	W	V	P	L	60
61	P	T	V	D	V	P	D	H	A	H	Y	T	L	G	T	V	I	L	L	V	80
81	G	L	T	G	M	L	G	N	L	T	V	I	Y	T	F	C	R	S	R	S	100
101	L	R	T	P	A	N	M	F	I	I	N	L	A	V	S	D	F	L	M	S	120
121	F	T	O	A	P	V	F	F	T	S	S	L	Y	K	Q	W	L	F	G	E	140
141	T	G	C	E	F	Y	A	F	C	G	A	L	F	G	I	S	S	M	I	T	160
161	L	T	A	I	A	L	D	R	Y	L	V	I	T	R	P	L	A	T	F	G	180
181	V	A	S	K	R	R	A	A	F	V	L	L	G	V	W	L	Y	A	L	A	200
201	W	S	L	P	P	F	F	G	W	S	A	Y	V	P	E	G	L	L	T	S	220
221	C	S	W	D	Y	M	S	F	T	P	A	V	R	A	Y	T	M	L	L	C	240

FIGURE 2B

241 C F V F F L P L L I I I Y C Y I F I F R 260  
261 A I R E T G R A L Q T F G A C K G N G E 280  
281 S L W Q R Q R L Q S E C K M A K I M L L 300  
301 V I L L F V L S W A P Y S A V A L V A F 320  
321 A G Y A H V L T P Y M S S V P A V I A K 340  
341 A S A I H N P I I Y A I T H P K Y R V A 360  
361 I A Q H L P C L G V L L G V S R R H S R 380  
381 P Y P S Y R S T H R S T L T S H T S N L 400  
401 S W I S I R R R Q E S L G S E S E V G W 420  
421 T H M E A A A V W G A A Q Q A N G R S L 440  
441 Y G Q G L E D L E A K A P P R P Q G H E 460  
461 A E T P G K T K G L I P S Q D P R M 478

5/35

100113192 10/018192

## FIGURE 3A

1 CAACTCAGGATGAACCCCTCCTTCGGGGCCAAAGAGTCCCGCCAGCCCAACCCAAGAGCCC 60

61 AGCTGCATGGCCACCCAGCACCAACCCAGCTGGTGGGACAGCTCCCAGAGCAGCATCTCC 120

121 AGCCTGGGCGGCTTCCATCATCATCAGTCCACAGCACCTGGGACTTGGGCTGCTGCCCTGG 180

181 GTCCCCCTCCCCACGGTTGATGTTCCAGACCAATGCCCACTATACCCCTGGGCACAGTGATC 240

241 TTGCTGGTGGGACTCACGGGGATGCTGGGCAACCTGACGGTCACTATATACCTTCTGTCAGA 300

301 GCTGTGCTTCGTGGAGTCACTGTGATGATGCAGAGCAGAAGCCCTCCGGACACCTTGCCCAAC 360

361 ATGTTCAATTATCAACCTCGCGGTCAGCGACTTCCTCATGTCCCTTACCCAGGCCCTGTC 420

421 TTCCTCACCAAGTACCTCTATAAGCAGTGGCTCTTTGGGGAGACAGGCTGCGAGTTCTAT 480

481 GCCTTCGTGGAGCTCTCTTTGGCATTTCCCTCCATGATCACCCCTGACGGCCATCGCCCTG 540

541 GACCGCTACCTGGTAATCACACGCCCGCTGGCCACCTTTGGTGTGGCGTCCAAGAGCGCT 600

601 GCGGCATTTGTCTCTGCTGGGCGTTTGGCTCTATGCCCTGGAGTCTGCCACCCCTTC 660

661 TTCGGCTGGAGCGCCTACGTGCCCGAGGGGTTGCTGACATCCTGCTCCTGGGACTACATG 720

6/35

FIGURE 3B

721 AGCTTCACGCCGGCGTGCGCTACACCATGCTTCTCTGCTGCTTCGTGTTCTTCCTC 780  
781 CCTCTGCTTATCATCATCTACTGCTACATCTTTCATCTTCAGGGCCATCCGGGAGACAGGA 840  
841 CGGGCTCTCCAGACCTTCGGGGCTGCAAGGGCAATGGCGAGTCCCTGTGGCAGCGGCAG 900  
901 CGGCTGCAGAGCGAGTGCAAGATGGCCAAAGATCATGCTGCTGGTCATCCTCCTCTTCGTG 960  
961 CTCTCCTGGGCTCCCTATTCCGCTGTGGCCCTGGTGGCCCTTTCGTGGGTACGCACACGTC 1020  
1021 CTGACACCCATCATGAGCTCGGTGCCAGCCGTCATCGCCAAGGCCTCTGCAATCCACAAC 1080  
1081 CCCATCATTTACGCCATCACCCACCCCAAGTACAGGTGGCCATTCGCCAGCACCTGCC 1140  
1141 TGCCCTGGGGTGCTGCTGGGTGTATCACGCCGGCACAGTCGCCCTACCCAGCTACCGC 1200  
1201 TCCACCCACCGCTCCACGCTGACCAGCCACACCTCCAACCTCAGCTGGATCTCCATACGG 1260  
1261 AGGCGCCAGGAGTCCCTGGGCTCGGAGAGTGAGGTGGGCTGGACACACATGGAGGCAGCA 1320  
1321 GCTGTGTGGGAGCTGCCCAGCAAGCAAATGGGCGGTCCCTCTACGGTCAGGGTCTGGAG 1380  
1381 GACTTGGAAGCCAAGGCACCCCCCAGACCCCCAGGACACGAAGCAGAGACTCCAGGGAAG 1440

WO 00/66630

PCT/US00/12065

8/35

FIGURE 3C

1441 ACCAAGGGGCTGATCCCCAGCCAGGACCCAGGATGTAGGACGCCCACTGGCTCTCCCTT 1500  
 1501 TCTTCTGAGACACATCCAGCCCCCCCCACGTCTCCCTCATAT 1541

WO 00/66630

PCT/US00/12065

FIGURE 4A

1 M N P P S G P R V P P S P T Q E P S C M 20  
 21 A T P A P P S W D S S Q S S I S S L G 40  
 41 R L P S I S P T A P G T W A A A W V P L 60  
 61 P T V D V P D H A H Y T L G T V I L L V 80  
 81 G L T G M L G N L T V I Y T F C R A V L 100  
 101 R G V T V M M Q S R S L R T P A N M F I 120  
 121 I N L A V S D F L M S F T O A P V F F T 140  
 141 S S L Y K Q W L F G E T G C E F Y A F C 160  
 161 G A L F G I S S M I T L T A I A L D R Y 180  
 181 L V I T R P L A T F G V A S K R R A A F 200  
 201 V L L G V W L Y A L A W S L P P F F G W 220  
 221 S A Y V P E G L L T S C S W D Y M S F T 240

9/35

10/35

241	P	A	V	R	A	Y	T	M	L	L	C	C	F	V	F	F	L	P	L	L	260
261	I	I	I	Y	C	Y	I	F	I	F	R	A	I	R	E	T	G	R	A	L	280
281	Q	T	F	G	A	C	K	G	N	G	E	S	L	W	Q	R	Q	R	L	Q	300
301	S	E	C	K	M	A	K	I	M	L	L	V	I	L	L	F	V	L	S	W	320
321	A	P	Y	S	A	V	A	L	V	A	F	A	G	Y	A	H	V	L	T	P	340
341	Y	M	S	S	V	P	A	V	I	A	K	A	S	A	I	H	N	P	I	I	360
361	Y	A	I	T	H	P	K	Y	R	V	A	I	A	Q	H	L	P	C	L	G	380
381	V	L	L	G	V	S	R	R	H	S	R	P	Y	P	S	Y	R	S	T	H	400
401	R	S	T	L	T	S	H	T	S	N	L	S	W	I	S	I	R	R	R	Q	420
421	E	S	L	G	S	E	S	E	V	G	W	T	H	M	E	A	A	A	V	W	440
441	G	A	A	Q	Q	A	N	G	R	S	L	Y	G	Q	G	L	E	D	L	E	460
461	A	K	A	P	P	R	P	Q	G	H	E	A	E	T	P	G	K	T	K	G	480

WO 00/66630

PCT/US00/12065

11/35

489

FIGURE 4C

481 L I P S Q D P R M



## FIGURE 5

1 CATAGCCATGGACCGCTATCTGGTGATCACACGTCCTCCACTGGCCACCATCGGCATGAGATC 60

61 CAAGAGACGGACGCACTAGTCCTGCTAGGTGCTGGCTCTATGCCCTGGCCTGGAGTCT 120

121 GCCGCCCTTCTTTGGCTGGAGCGCCTACGTGCCCCGAGGGGCTGCTGACATCCTGCTCCTG 180

181 GGA CTACGTGACCTTACGCGCCCCCTCGTGCGCGCCCTACACCATGCTGCTCTTCTGCTTTGT 240

241 CTTCTTCCTC 250

12/35

13/35

FIGURE 6

1	<u>I A M</u>	D R Y L V L V L L G V W L Y A L A W S L	20
21	K R R T A L V L L G V W L Y A L A W S L	40	
41	<u>P P F F</u>	G W S A Y V P E G L L T S C S W	60
61	D Y V T F T P L V R A Y T M L L F C F V	80	
81	<u>F F L</u>	83	

WO 00/66630

10/01/02

PCT/US00/12065

14/35

NOT FURNISHED UPON FILING

15/35

FIGURE 7B

Rat SNORF36	163	GCTGACATCCTGCTCCTGGGACTACGTGACCTTCACGCCCTCGTGCGCG	212
Hum SNORF36	651	GCTGACATCCTGCTCCTGGGACTACATGAGCTTCACGCCGCGCGTG	700
Rat SNORF36	213	CCTACACCATGCTGCTCTTCTGCTTGTCTTCTTCCTC	250
Hum SNORF36	701	CCTACACCATGCTTCTCTGCTGCTTGTGTTCTTCTCCCTCTGCTTATC	750

16/35

FIGURE 8

Rat SNORF36	1	.....IAMDRLVITRPLATIGMRSKRRRTALVLLGVWLYALA	37
		:      .	
Hum SNORF36	151	ALFGISSMITLTAIALDRLVITRPLATFGVASKRRAAFVLLGVWLYALA	200
Rat SNORF36	38	WSLPPFFGWSAYVPEGLLTSCSWDYVTFPLVRAYTMLLFCFVFFL....	83
		.	
Hum SNORF36	201	WSLPPFFGWSAYVPEGLLTSCSWDYMSFTPAVRAYTMLLCCFVFFLPLLI	250

100 111 541 10/01/92

## FIGURE 9A

17/35

1	TTTAAGTCCTCCAAGAGCCTGAGCATGA <u>ACTCTCCTTC</u> CAGAAATCAAGAGTCCCTTCAAGC	60
61	TTAACTCAGGATCCCAGCTTTACCGCCAGCCCTGCCCTCCTACAAGGCATTTGGAAACAGC	120
121	ACTCAGAACATCTCCGTGAGAGTCCAGCTTCTATCCGTTAGCCCCACGACACCTGGGCTT	180
181	CAGGCTGCTGCCCTGGTCCCCTTCCCCACAGTCGACGTCCCAGATCATGCTCACTATACC	240
241	CTAGGCACGGTGATCCTGCTGGTGGGACTCACAGGGATGCTGGGTAACCTGACAGTCATC	300
301	TACACCTTCTGCAGGAATAGAGGCCTGCGGACACCCGGCAACATGCTCATCAACCTG	360
361	GCAGTCAGCGACTTCCTTATGTCGTTCACTCAGGCCCCGGTCTTCTTTGCCAGCAGCCTC	420
421	TACAAGAAGTGGCTCTTCGGGGAGACAGGTTGCAAGTTCTATGCCCTTCTGTGGGGCTGTC	480
481	TTTGGCATCGTTTCCATGATCACCCCTGACAGCCCATAGCCATGGACCGCTATCTGGTGATC	540
541	ACACGTCCACTGGCCACCATCGGCATGAGATCCAAGAGACGGACGGCACTAGTCCTGCTA	600
601	GGTGCTGGCTCTATGCCCTGGCCCTGGAGTCTGCCGCCCTTCTTTGGCTGGAGCGCCTAC	660

FIGURE 9B

18/35

661 GTGCCCAGGGGCTGCTGACATCCTGCTCCTGGACTACGTGACCTTCACGCCCTCGTG 720  
721 CGCGCCTACACCATGCTGCTCTTCTGCTTTGTCTTCTTCCCTCCCTCTGCTCATATCATC 780  
781 TTCTGCTACATCTTCATCTTCAGGGCCATTTCGAGAGACAGCGCGGCTGTGAGGGCTGT 840  
841 GGTGAGTCCCCCTCTGCGGGGGGCGAGTGGCAGCGGCTACAGAGTGAATGGAAGATGGCC 900  
901 AAGGTGCGCACTGATCGTCAATTCTCCTCTTTGTGCTGTCCCTGGGCTCCCTACTCCACTGTG 960  
961 GCCCTGGTGGGCTTTGCTGGGTACTCGGCACATCCTGACGCCCTACATGAGCTCGGTGCCA 1020  
1021 GCCGTCAATTGCCAAGGCCCTCGGCCCATCCACAATCCTATCATATGCCATCACTCACCCCC 1080  
1081 AAGTACAGGGCGGCCATTGCTCAGCACTTGCCCTTGCCCTTGGGGTGCTTCTTGAGTATCA 1140  
1141 GGCAGCGCAGCCACCCCTCCCTCAGCTACCGCTCTACCCATCGCTCCACACTGAGCAGC 1200  
1201 CAGTCCCTCAGACCTCAGCTGGATCTCTGGGCAGAGCGCCCAAGAGTCCCTGGGTTCTGAG 1260  
1261 AGTGAAGTGGGCTGGACAGACACAGAAACAACAGCTGCGTGGGGAGCTGCCCCAGCAAGCA 1320

10/018192

19/35

## FIGURE 9C

1321 AGTGGACAATCCTTCTGCAGTCATGACCTGGAAGATGGAGAAGTCAAGGCTCCTTCCAGC 1380  
1381 CCCCAGGAACAGAAATCCAAGACTCCCAAGACCAAGAGACACCTCCCCAGTCTGGACCGA 1440  
1441 AGGATGTAGGATGCCCCAGTCCCGTCCCCCTCCCT 1473



10/01/92

20/35

FIGURE 10A

1	M	N	S	P	S	E	S	R	V	P	S	S	L	T	Q	D	P	S	F	T	20
21	A	S	P	A	L	L	Q	G	I	W	N	S	T	Q	N	I	S	V	R	V	40
41	Q	L	L	S	V	S	P	T	T	P	G	L	Q	A	A	A	W	V	P	F	60
61	P	T	V	D	V	P	D	H	A	H	Y	T	L	G	T	V	I	L	L	V	80
81	G	L	T	G	M	L	G	N	L	T	V	I	Y	T	F	C	R	N	R	G	100
101	L	R	T	P	A	N	M	L	I	I	N	L	A	V	S	D	F	L	M	S	120
121	F	T	Q	A	P	V	F	F	A	S	S	L	Y	K	K	W	L	F	G	E	140
141	T	G	C	K	F	Y	A	F	C	G	A	V	F	G	I	V	S	M	I	T	160
161	L	T	A	I	A	M	D	R	Y	L	V	I	T	R	P	L	A	T	I	G	180
181	M	R	S	K	R	R	T	A	L	V	L	L	G	V	W	L	Y	A	L	A	200
201	W	S	L	P	P	F	F	G	W	S	A	Y	V	P	E	G	L	L	T	S	220

21/35

**SUBSTITUTE SHEET (RULE 26)**

22/35

FIGURE 10C

441	D	L	E	D	G	E	V	K	A	P	S	S	P	Q	E	Q	K	S	K	T	460
461	P	K	T	K	R	H	L	P	S	L	D	R	R	M							474

**SUBSTITUTE SHEET (RULE 26)**

24/35

FIGURE 11B

hSNORF36a	401	AGCAGTGGCTCTTTGGGGAGACAGGCTGCGAGTTCTATGCCCTTCTGTGGA	450
rSNORF36	401	AGAAAGTGGCTCTTCGGGGAGACAGGTTGCAAGTTCTATGCCCTTCTGTGGG	450
hSNORF36a	451	GCTCTCTTTGGCATTTCTCCATGATCACCCCTGACGGCCATGCGCCCTGGA	500
rSNORF36	451	GCTGTCTTTGGCATCGTTTCCATGATCACCCCTGACAGCCATAGCCATGGA	500
hSNORF36a	501	CCGCTACCTGGTAATCACACGCCCGCTGGCCACCTTTGGTGTGGCGTCCA	550
rSNORF36	501	CCGCTATCTGGTGATCACACGCTCCACTGGCCACCATCGGCATGAGATCCA	550
hSNORF36a	551	AGAGCGGTGCGGCATTTGTCTCTGCTGGCGTTTGGCTCTATGCCCTGGCC	600
rSNORF36	551	AGAGACGGACGGCACTAGTCCTGCTAGGTGCTGGCTCTATGCCCTGGCC	600
hSNORF36a	601	TGGAGTCTGCCACCCCTTCTTCGGCTGGAGCGCTACGTGCCCGAGGGGTT	650
rSNORF36	601	TGGAGTCTGCCCGCCCTTCTTTGGCTGGAGCGCCCTACGTGCCCGAGGGGCT	650
hSNORF36a	651	GCTGACATCCTGCTCCTGGGACTACATGAGCTTCACGCCCGGCCGTCGCTG	700
rSNORF36	651	GCTGACATCCTGCTCCTGGGACTACGTGACCTTCACGCCCTCCTGTCGCGG	700
hSNORF36a	701	CCTACACCATGCTTCTCTGCTGCTTCGTGTTCTTCTCCTCCCTCTGCTTATC	750
rSNORF36	701	CCTACACCATGCTGCTCTCTGCTTGTCTTCTTCTCCTCCCTCTGCTCATT	750
hSNORF36a	751	ATCATCTACTGCTACATCTTCATCTTTCAGGGCCATCCGGGAGACAGGACG	800
rSNORF36	751	ATCATCTTCTGCTACATCTTTCATCTTTCAGGGCCATTTCGAGAGACAGGCCG	800

25/35

FIGURE 11C

hSNORF36a	801	GGCTCTCCAGACCTTCGGGGCCCTGCAAGGGCAATGGCGAGTCCCTGTGGC	850
rSNORF36	801	GG.....CCTGTAGGGCTGTGTGAGTCCCTCTGCGGCGGCGGC	841
hSNORF36a	851	AGCGGCAGCGGCTGCAGAGCGAGTGCAAGATGGCCAAAGATCATGCTGCTG	900
rSNORF36	842	AGTGGCAGCGGCTACAGAGTGAATGGAAGATGGCCAAAGTGCACATGATC	891
hSNORF36a	901	GTCATCCTCCTCTTCGTGCTCTCCTGGGCTCCCTATTCCGCTGTGGCCCT	950
rSNORF36	892	GTCATTCTCCTCTTGTGCTGTCTCTGGGCTCCCTACTCCACTGTGGCCCT	941
hSNORF36a	951	GGTGGCCTTTGCTGGGTACGCACACGTCCTTGACACCCCTACATGAGCTCGG	1000
rSNORF36	942	GGTGGCCTTTGCTGGGTACTCGCACATCCTGACGCCCTACATGAGCTCGG	991
hSNORF36a	1001	TGCCAGCCGTCTCGCCAAAGGCCTCTGCAATCCACAACCCCATCATTTAC	1050
rSNORF36	992	TGCCAGCCGTCTCGCCAAAGGCCTCGGCCATCCACAATCCTATCATCTAT	1041
hSNORF36a	1051	GCCATCACCCACCCCAAGTACAGGGTGGCCATTGCCCCAGCACCTGCCCTG	1100
rSNORF36	1042	GCCATCACTCACCCCAAGTACAGGGGGGCCATTGCTCAGCACTTGCCCTTG	1091
hSNORF36a	1101	CCTGGGGGTGCTGTTGGTGTATCAGCCGGGACAGTCGCCCCCTACCCCA	1150
rSNORF36	1092	CCTGGGGGTGCTTCTGGAGTATCAGGCCAGCGCAGCCACCCCTCCCTCA	1141
hSNORF36a	1151	GCTACCGCTCCACCCACCGCTCCACGCTGACCAGCCACACCTCCAAACCTC	1200
rSNORF36	1142	GCTACCGCTCTACCCCATCGCTCCACACTGAGCAGCCAGTCTCTCAGACCTC	1191

26/35

FIGURE 11D

hSNORF36a	1201	AGCTGGATCTCCATACGGAGGCGCCAGGAGTCCCTGGGCTCGGAGAGTGA	1250
rSNORF36	1192	AGCTGGATCTCTGGGCAGAAGCGCCCAAGAGTCCCTGGGTTCTGAGAGTGA	1241
hSNORF36a	1251	GGTGGGCTGGACACACATGGAGGCAGCAGCTGTGTGGGAGCTGCCCCAGC	1300
rSNORF36	1242	AGTGGGCTGGACAGACACAGAAACAACAGCTGCGTGGGAGCTGCCCCAGC	1291
hSNORF36a	1301	AAGCAAATGGGCGGTCCCTCTACGGTCAGGGTCTGGAGGACTTGGAAAGCC	1350
rSNORF36	1292	AAGCAAGTGGACAATCCTTCTGCAGTCATGACCTGGAAGATGGAGAAGTC	1341
hSNORF36a	1351	AAGGCACCCCCCAGACCCCCAGGGACACGAAGCAGAGACTCCAGGGAAGAC	1400
rSNORF36	1342	AAGGCTCCTTCCAGCCCCCAGGAACAGAAATCCAAGACTCC...CAAGAC	1388
hSNORF36a	1401	CAAGGGGCTGATCCCCCAGCCAGGACCCCCAGGATGTAG	1437
rSNORF36	1389	CAAGAGACACCTCCCCCAGTCTGGACCCGAAGGATGTAG	1425

**FIGURE 12A**

hSNORF36a	1	MNPPSGPRVPPSPTQEPSCMATPA.PPSWWDSSQSIISSLGRLPISIPTA	49
rSNORF36	1	MNSPSESrvPSSLTQDPSTASPALLQGIWNSTQ.NISVRVQLLSVSPTT	49
hSNORF36a	50	PGTWAAAwwPLPTVDVPDHAHYTLGTvILLVGLTGMLGNLTVIYTFCSR	99
rSNORF36	50	PGLQAAAwwPFPTVDVPDHAHYTLGTvILLVGLTGMLGNLTVIYTFCRNR	99
hSNORF36a	100	SLRTPANMFIINLAVSDFLMSFTQAPVFFTSSLYKQWLFGETGCEFYAFC	149
rSNORF36	100	GLRTPANMLIINLAVSDFLMSFTQAPVFFASSLYKKWLFGETGCKFYAFC	149
hSNORF36a	150	GALFGISSMITLTAIALDRYLVTITRPLATFGVASKRRAAAFVLGGVWLYAL	199
rSNORF36	150	GAVFGIVSMITLTAIAMDRYLVTITRPLATIGMRSKRRRTALVLLGVWLYAL	199
hSNORF36a	200	AWSLPFFFGSAYVPEGLLTSCSWDYMSFTPAVRAYTMLLCCFVFFLP LL	249
rSNORF36	200	AWSLPFFFGSAYVPEGLLTSCSWDYVTFPTPLVRAYTMLLCFVFFLP LL	249
hSNORF36a	250	IIICYIFIFRAIRETGRALOTFGACKNGESLWOR...QRLOSECKMAK	296
rSNORF36	250	IIIFYCIFIRAIRETR.....ACEGCGESPLRRRQQRLQSEWKMAK	293
hSNORF36a	297	IMLLVILLFVLSWAPYSAVALVAFAGYAHLTPYMSSVPAVIKASAIHN	346
rSNORF36	294	VALIVILLFVLSWAPYSTVALVGAGYSHILTPYMSSVPAVIKASAIHN	343
hSNORF36a	347	PIIYAITHPKYRVIAIAQHLPCLGVLLGVSRHRHSPYPYSRSTHRTLTS	396
rSNORF36	344	PIIYAITHPKYRAAIAQHLPCLGVLLGVSGQRSHPSLSYRSTHRTLSSQ	393



**FIGURE 12B**

hSNORF36a	397	TSNLSWISIRRRQESLGESEVGTWMEAAA	VWGAQQANGRSLYQGGL	446
		. . . . . . . . . . . . . . . . .		
rSNORF36	394	SSDLSWISGQKRQESLGESEVGTDTET	TAAWGAQQASGQSFCSHDLE	443
hSNORF36a	447	DLEAKAPRRPQGHEAETPGTKGLIPSQDPRM		478
		. . . . . . . . . . . . . . . .		
rSNORF36	444	DGEVKAPSSPQEQKSKTP.KTKRHLP	SLDRRM	474

29/35

FIGURE 13A

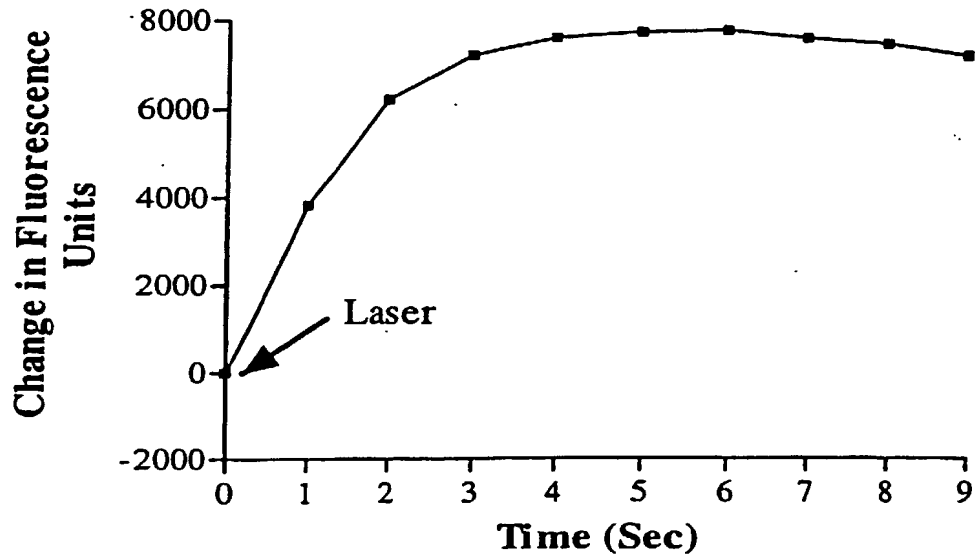
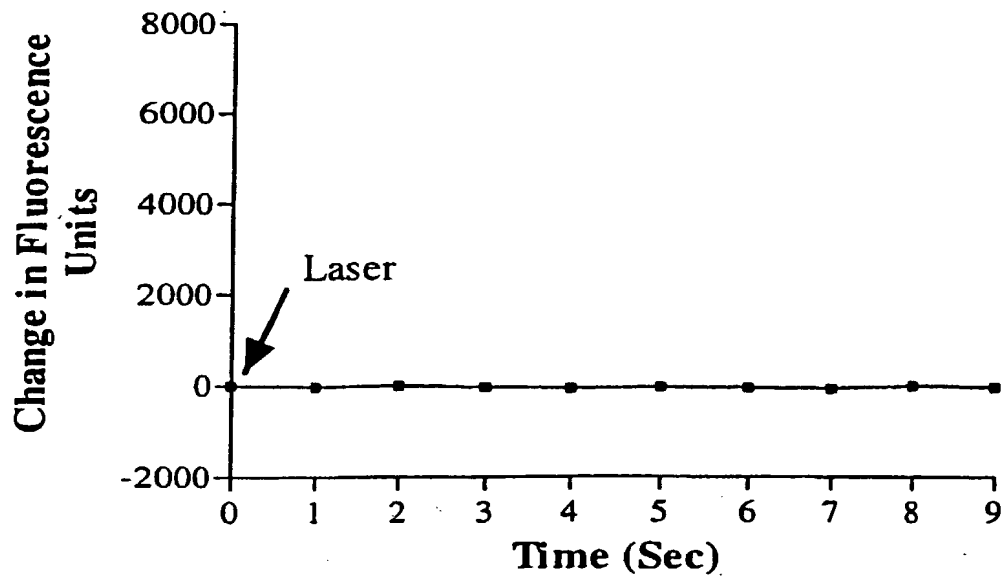
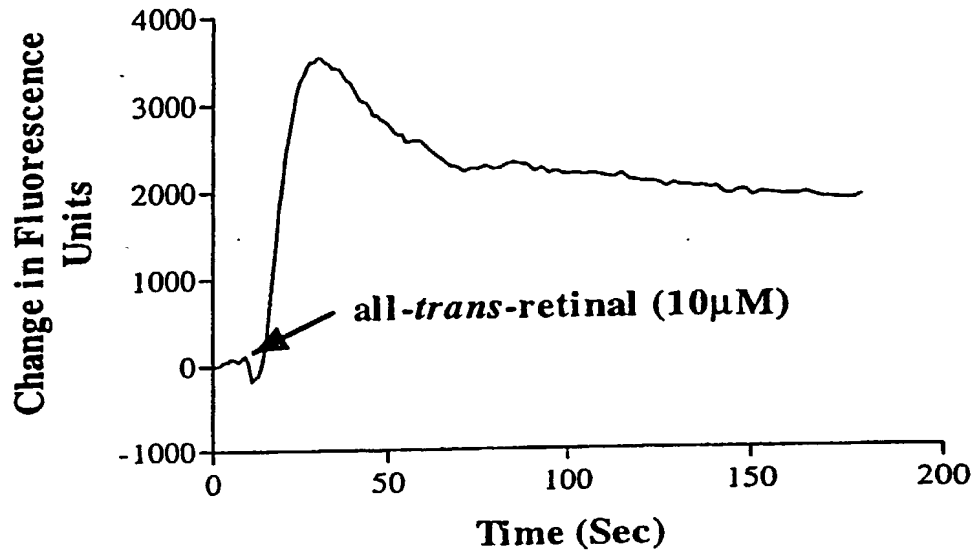


FIGURE 13B

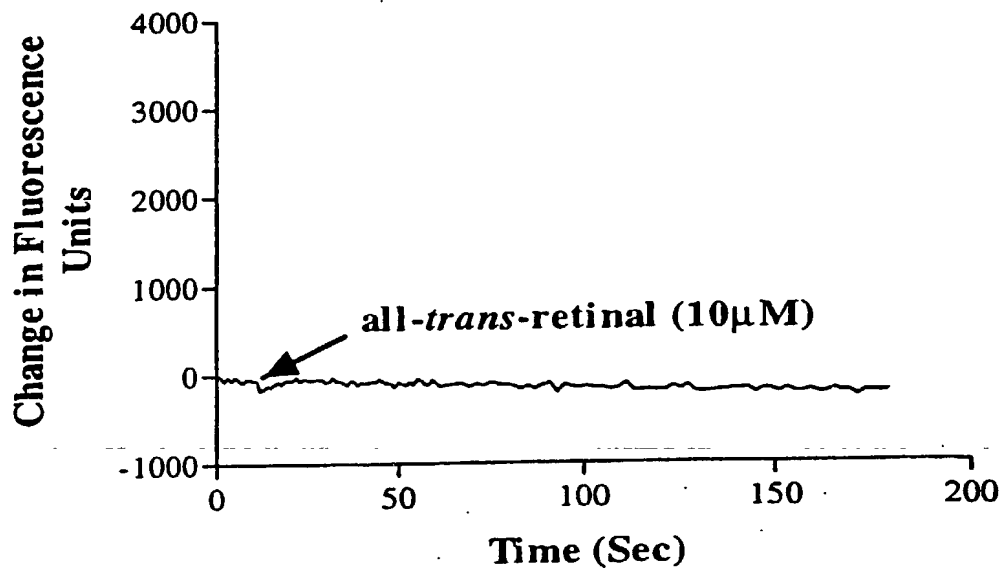


30/35

**FIGURE 14A**



**FIGURE 14B**



31/35

FIGURE 15A

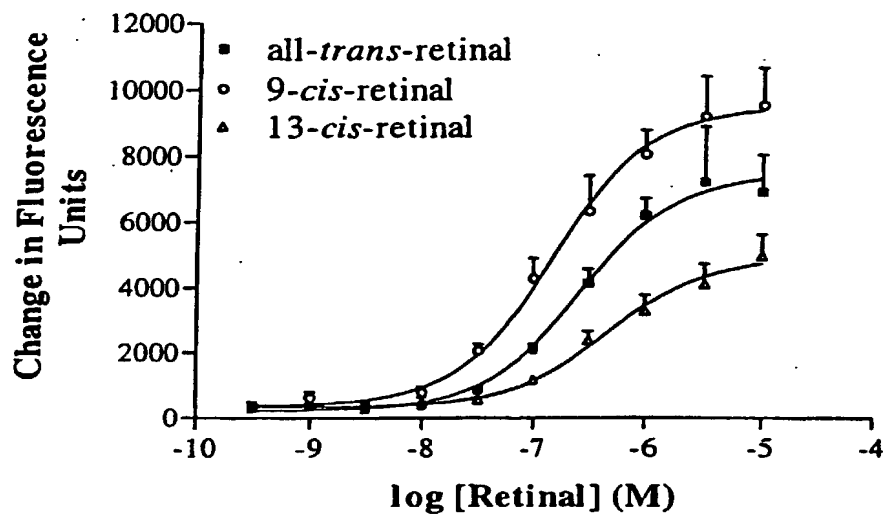
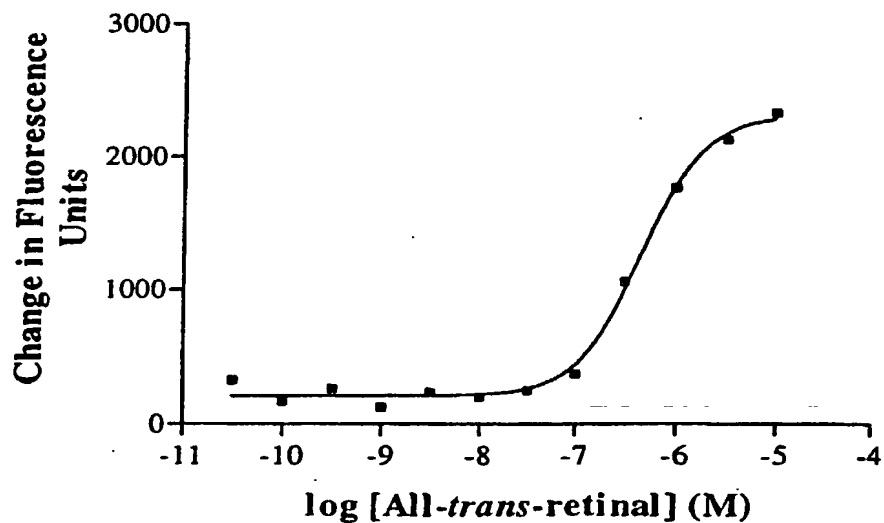


FIGURE 15B



32/35

FIGURE 16A

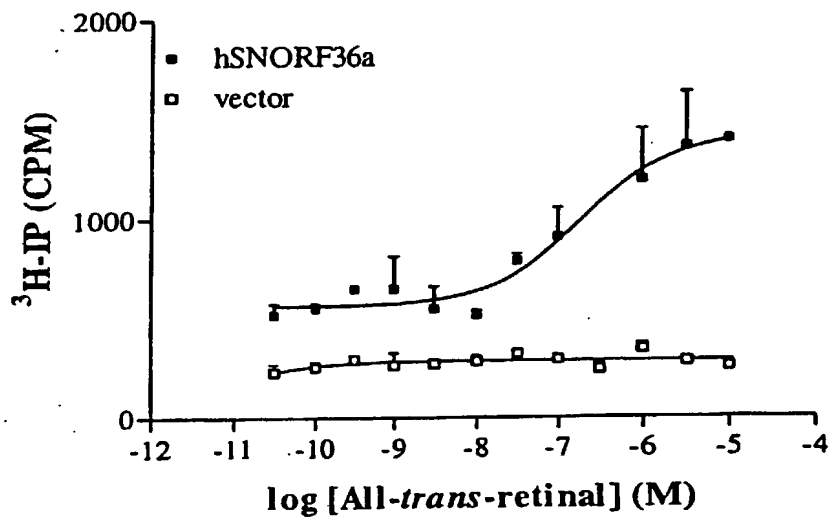
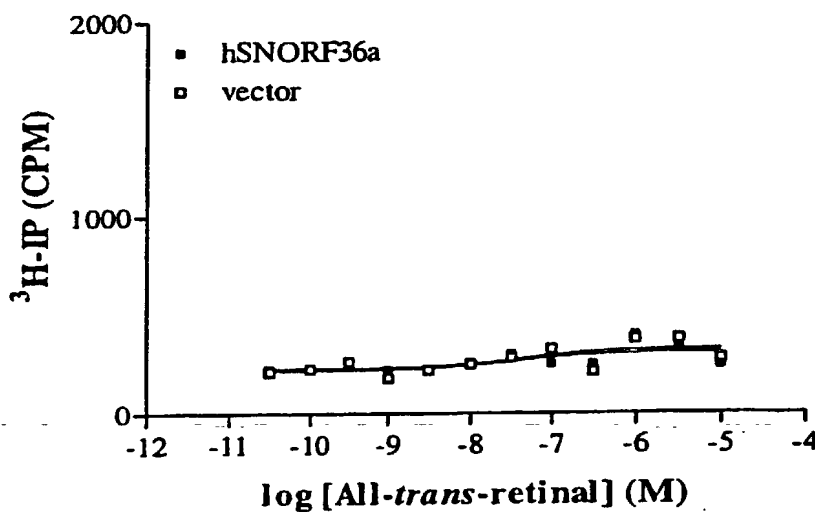


FIGURE 16B



33/35

FIGURE 17A

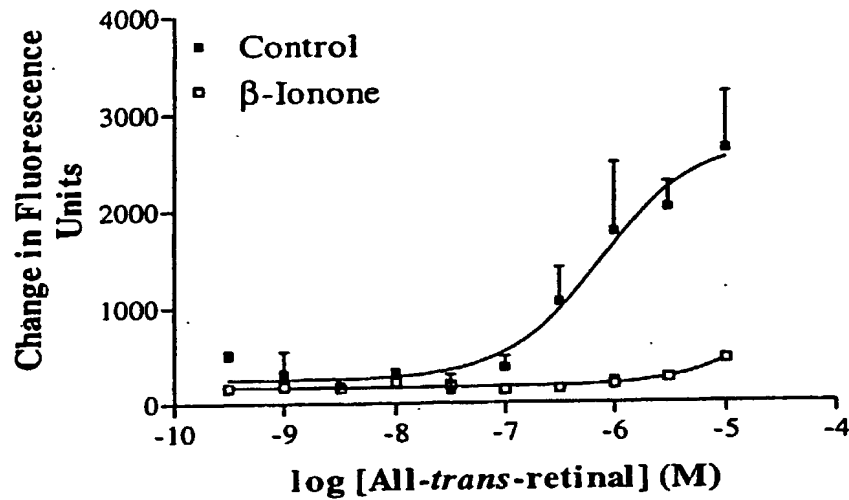
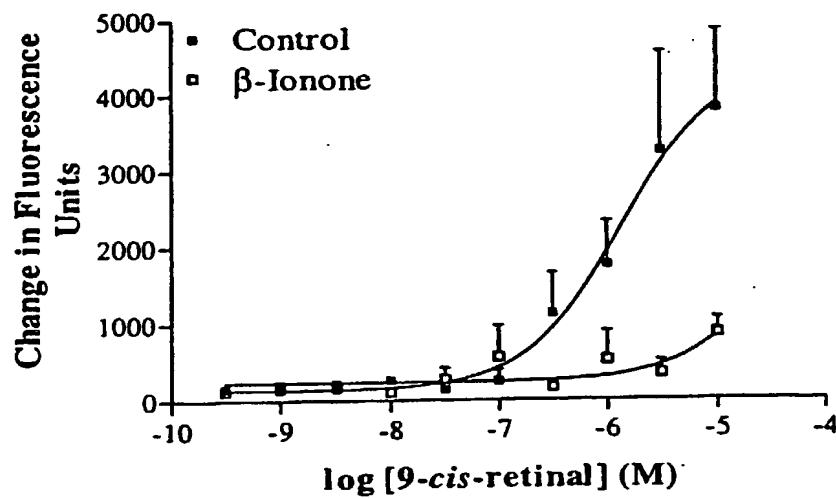


FIGURE 17B



34/35

FIGURE 18A

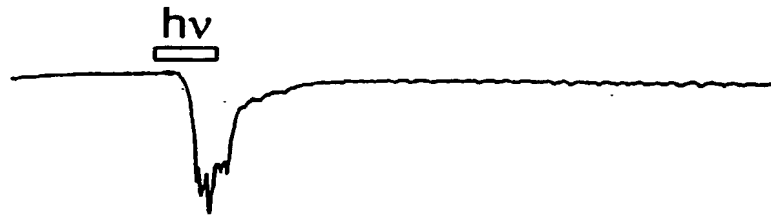


FIGURE 18B

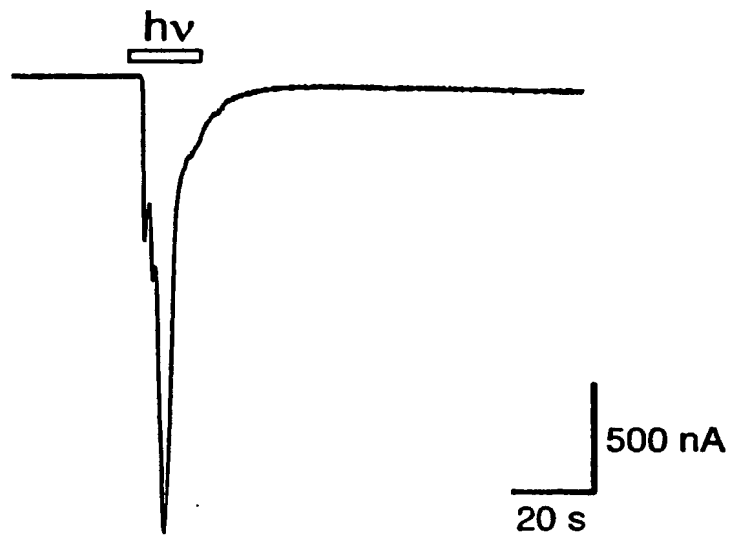
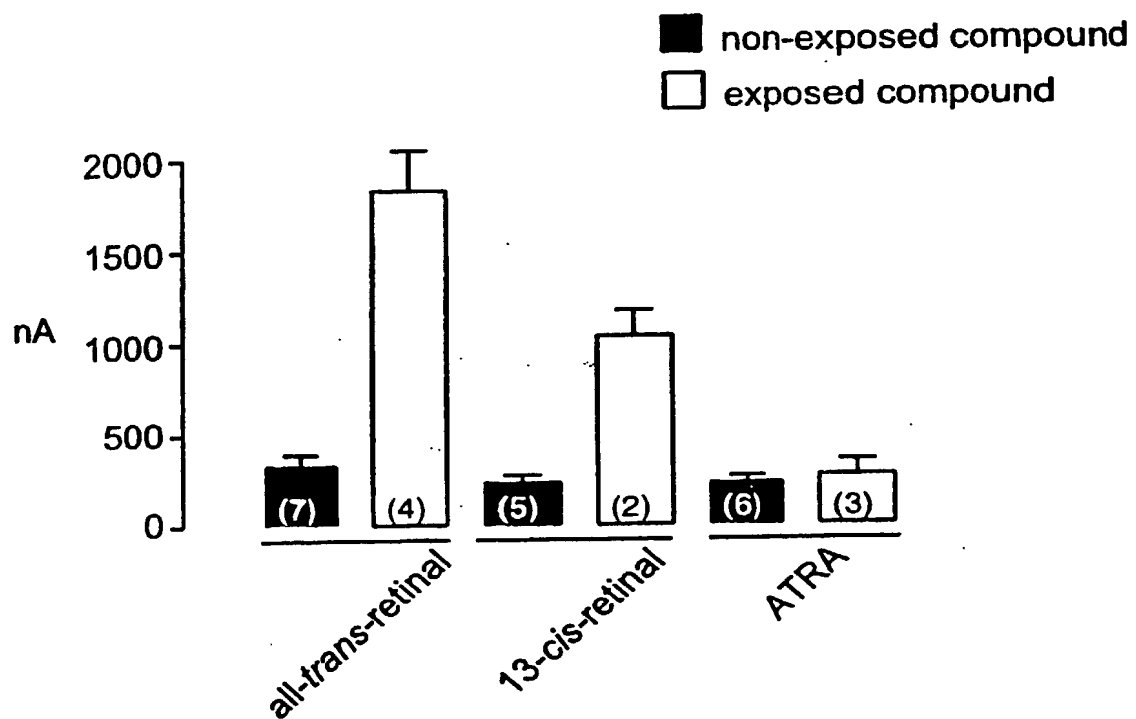


FIGURE 18C



35/35

Figure 19





**DECLARATION AND POWER OF ATTORNEY**

*As a below-named inventor, I hereby declare that:*

*My residence, post office address, and citizenship are as stated below next to my name.*

*I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:*

*the specification of which:  
(check one)*

       *is attached hereto.*

  X   *was filed on* November 1, 2001 *as*

*Application Serial No.* 10/018,192

*and was amended* November 1, 2001

*(if applicable)*

*I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.*

*I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.*

*I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 (a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International Application which designated at least one country other than the United States, listed below. I have also identified below any foreign application for patent or inventor's certificate, or PCT International Application having a filing date before that of the earliest application from which priority is claimed:*

<i>Prior Foreign Application(s)</i>			<i>Priority Claimed</i>	
<u>Number</u>	<u>Country</u>	<u>Filing Date</u>	<u>Yes</u>	<u>No</u>
PCT/US00/12065	PCT	3 May 2000	<u>  X  </u>	<u>      </u>
<u>          </u>	<u>          </u>	<u>          </u>	<u>      </u>	<u>      </u>
<u>          </u>	<u>          </u>	<u>          </u>	<u>      </u>	<u>      </u>
<u>          </u>	<u>          </u>	<u>          </u>	<u>      </u>	<u>      </u>
<u>          </u>	<u>          </u>	<u>          </u>	<u>      </u>	<u>      </u>
<u>          </u>	<u>          </u>	<u>          </u>	<u>      </u>	<u>      </u>

Applicants: Beth E. Borowsky et al.  
U.S. Serial No.: 10/018,192  
Entered U.S. National Stage:  
November 1, 2001  
**Exhibit B**

*Declaration and Power of Attorney*

Page 2

*I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below:*

<u>Provisional Application No.</u>	<u>Filing Date</u>	<u>Status</u>
N/A		

*I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States Application(s), or Section 365(c) of any PCT International Application(s) designating the United States listed below. Insofar as this application discloses and claims subject matter in addition to that disclosed in any such prior Application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56, which became available between the filing date(s) of such prior Application(s) and the national or PCT international filing date of this application:*

<u>Application Serial No.</u>	<u>Filing Date</u>	<u>Status</u>
09/518,914	3 March 2000	Pending
09/303,593	3 May 1999	Abandoned
PCT/US00/12065	3 May 2000	Pending

*And I hereby appoint*

*John P. White (Reg. No. 28,678); Christopher C. Dunham (Reg. No. 22,031); Norman H. Zivin (Reg. No. 25,385); Jay H. Maioli (Reg. No. 27,213); William E. Pelton (Reg. No. 25,702); Robert D. Katz (Reg. No. 30,141); Peter J. Philips (Reg. No. 29,691); Wendy E. Miller (Reg. No. 35,615); Richard S. Milner (Reg. No. 33,970); Roberto T. Maldonado (Reg. No. 38,232); Paul Teng (Reg. No. 40,837); Richard F. Jaworski (Reg. No. 33,515); Pedro C. Fernandez (Reg. No. 41,741); Gary J. Gershik (Reg. No. 39,992); Spencer H. Schneider (Reg. No. 45,923); Alan J. Morrison (Reg. No. 37,399); Alan D. Miller (Reg. No. 42,889); and Frank Bruno (Reg. No. 46,583)*

*and each of them, all c/o Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, New York 10036, my attorneys, each with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications which are based thereon under the provisions of the Patent Cooperation Treaty.*

Declaration and Power of Attorney

Page 3

Please address all communications, and direct all telephone calls, regarding this application to:

John P. White

Reg. No. 28,678

Cooper & Dunham LLP  
1185 Avenue of the Americas  
New York, New York 10036  
Tel. (212) 278-0400

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

100 Full name of sole or first joint inventor Beth E. Borowsky

Inventor's signature Beth E. Borowsky

Citizenship United States of America Date of signature 11/5/01

Residence 218 Park Street, Montclair, New Jersey 07042

Post Office Address same as above NJ

200 Full name of joint inventor (if any) Kristine L. Ogozalek

Inventor's signature Kristine L. Ogozalek

Citizenship United States of America Date of signature 11/5/01

Residence 25 Durand Place, Rochelle Park, New Jersey 07662

Post Office Address same as above NJ

Full name of joint inventor (if any) Parul P. Lakhani

Inventor's signature \_\_\_\_\_

Citizenship India Date of signature \_\_\_\_\_

Residence 1020 The Lane, Lexington, Kentucky 40504

Post Office Address same as above

***Declaration and Power of Attorney***

**Page 3**

*Please address all communications, and direct all telephone calls, regarding this application to:*

**John P. White**

Reg. No. 28,678

**Cooper & Dunham LLP**  
1185 Avenue of the Americas  
New York, New York 10036  
Tel. (212) 278-0400

*I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.*

**Full name of sole or first joint inventor** Beth E. Borowsky

*Inventor's signature*

**Citizenship** United States of America **Date of signature** \_\_\_\_\_

Residence 218 Park Street, Montclair, New Jersey 07042

*Post Office Address* same as above

Full name of joint inventor (if any) Kristine L. Ogozalek

***Inventor's signature.***

Citizenship United States of America Date of signature

Residence 25 Durand Place, Rochelle Park, New Jersey 07662

Post Office Address same as above

Full name of joint inventor (if any) Parul P. Lakhiani

***Inventor's signature.***

Citizenship India

Date of signature 6/17/02

Residence 328 Atwood Drive, Lexington, Kentucky 40515

Post Office Address same as above

